

BERICHTE  
aus dem  
INSTITUT FÜR MEERESKUNDE  
an der  
CHRISTIAN-ALBRECHTS-UNIVERSITÄT KIEL

Nr.329

DOI 10.3289/IFM-BER-329

A comparison of the impact of major mesozooplankton  
taxa on marine, brackish and freshwater phytoplankton  
during summer

by  
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Kiel 2003

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Kopien dieser Arbeit können bezogen werden durch:

Institut für Meereskunde  
-Bibliothek-  
Düsternbrooker Weg 20  
D-24105 Kiel  
Germany



ISSN 0341-8561

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## Zusammenfassung

Das herbivore Zooplankton stellt die wichtigste trophische Verbindung zwischen dem Phytoplankton und Fischen in marinen wie auch limnischen Systemen dar. Herbivore Zooplankter unterscheiden sich in ihrer Körper-Stöchiometrie und in der Art, wie sie Nahrung filtrieren. Copepoden sind fähig, Partikel zu greifen und können daher aktiv große Algen und Mikrozooplankter selektieren. Sie sind relativ reich an Stickstoff und zeichnen sich daher durch ein hohes Stickstoff zu Phosphor (N:P) Verhältnis aus. Mechanische Filtrierer (z. B. Süßwasser-Cladoceren und Appendicularien) sind dagegen - was die Beutegröße betrifft - eher auf kleinere Partikel beschränkt. Sie sind zudem relativ reich an Phosphor und haben daher ein niedriges N:P Verhältnis. Von diesen Grundlagen ausgehend, wurden die folgenden Hypothesen formuliert: (1) Copepoden verschieben die Größenverteilung von Phytoplankton-Gemeinschaften in Richtung kleiner Partikelgrößen, indem sie große Phytoplankter, aber auch Mikrozooplankter entfernen. Mechanische Filtrierer ingestieren Partikel kleinerer Größe und fördern daher das Wachstum großer Phytoplankter. (2) Als Konsequenz, ist das Stabile Isotopen-Signal von Stickstoff ( $\delta^{15}\text{N}$ ) - ein Indikator für trophische Anreicherung - bei Copepoden höher als bei mechanischen Filtrierern, weil das Mikrozooplankton einen höheren Anteil an der Gesamtnahrung einnimmt. (3) Beruhend auf theoretische Überlegungen der Ökologischen Stöchiometrie, verursachen Zooplankter mit einem hohen Körper-N:P Verhältnis Stickstoff (N)-Limitation beim Phytoplankton, während Zooplankter mit einem niedrigen Körper-N:P Verhältnis das Gegenteil, also Phosphor (P)-Limitation verursachen. Dies geschieht, weil das Element, wofür der Bedarf am Höchsten ist, bevorzugt zurückgehalten und das Element im Überschuss ausgeschieden wird.

Diese Hypothesen wurden in drei Mesokosmos-Versuchen getestet, und zwar (1) im Schöhsee, Norddeutschland im August 2000, (2) in einer norwegischen Meereslagune (Hopavågen) im Juli 2001, und (3) in der Kieler Förde, Westliche Ostsee, im September 2003. An allen drei Orten wurde ein Copepoden-Treatment bestehend aus einem logarithmisch skalierten Gradienten von Copepoden-Dichten (5 bis 80 oder 160 ind  $\text{L}^{-1}$ ) geschaffen. Die Copepoden wurden der natürlichen Copepoden-Gemeinschaft vor Ort entnommen. Im Schöhsee-Experiment bestand das Cladoceren-Treatment - ähnlich wie das Copepoden-Treatment - aus einem logarithmisch skalierten Dichte-Gradienten der im Labor gezüchteten Cladocere *Daphnia hyalina x galeata* (2.5 bis 40 ind  $\text{L}^{-1}$ ). An beiden marinen Standorten wurden in einigen Säcken zunächst Copepoden entfernt, von man glaubt, dass sie juvenile Appendicularien oder deren Eier fressen. In diesen so genannten Appendicularien-Treatments entwickelte sich im Hopavågen tatsächlich die Appendicularie *Oikopleura dioica* bis zu einer Maximal-Dichte von 35 ind  $\text{L}^{-1}$ . Im Kieler Förde-Experiment ist dies nicht geglückt. Die Mesokosmen (~1.5 oder ~3.4  $\text{m}^3$  Volumen) wurden im Abstand von 3 bis 4 Tagen für Phytoplankton- und Zooplanktonzählungen, sowie für die Bestimmung der Kohlenstoff (C), N und P-Konzentrationen von vorfiltriertem (<64 oder <100  $\mu\text{m}$ ), partikulärem organischen Material - hier kurz als 'Seston' bezeichnet - beprobt. Proben für die Bestimmung des  $\delta^{15}\text{N}$  von Zooplankton und Seston wurden zu

Anfang und am Ende der marinen Experimente - im Schöhsee-Experiment nur am Ende für Zooplankton - genommen.

Das Schöhsee-Phytoplankton war sehr artenreich. Copepoden zeigten eine negative Wirkung auf große ( $>4000 \mu\text{m}^3$ ) Phytoplankter, während Cladoceren kleine Phytoplankter unterdrückten. Sie waren somit komplementär in ihrem Einfluss auf die Größe von Phytoplankton-Arten. Im Nahrungsnetz des Hopavågen stellten heterotrophe Ciliaten einen dominanten Anteil dar. Copepoden zeigten auch hier eine negative Wirkung auf große Partikel (Diatomeen, Ciliaten) und eine positive auf kleine ( $<5 \mu\text{m}$ ) Nanoflagellaten, wahrscheinlich über eine trophische Kaskade. Für Appendicularien wurden keine Korrelationen gefunden. Im Experiment in der Kieler Förde war die Phytoplankton-Gemeinschaft ebenfalls artenreich. Allerdings, gab es auf ähnlich große Algen gleichermaßen positive wie negative Effekte. Beachtenswert war, dass die diazotrophe Blaualge *Nodularia spumigena* in allen Mesokosmen in ihrer Abundanz zunahm. Während Copepoden kurzzeitig (3 Tage) einen negativen Effekt auf die Konzentration von *N. spumigena* Zellen hatte, waren keine Langzeit-Effekte (9 Tage) auf Zell-Konzentrationen oder auf die Größenfrequenz-Verteilung der Trichome (als Indikator für 'filament clipping') ersichtlich. Bezüglich der ersten Hypothese, kann also die Schlussfolgerung gezogen werden, dass Süßwasser-Copepoden und Cladoceren tatsächlich das Größenspektrum von Phytoplankton-Arten zugunsten von großen bzw. kleinen Algen verschieben können. In oligotrophen marinen Systemen (Hopavågen) scheinen Copepoden Phytoplankter ebenfalls auf der Basis von ihrer Größe zu selektieren, während sie unter meso- bis eutrophen Bedingungen (Kieler Förde) vielleicht stärker auf den 'Geschmack' der Algen (Diatomeen) achten.

Da diazotrophe Blaualgen sowohl im Schöhsee (*Anabaena flos-aquae*, *Microcystis* sp.) als auch in der Kieler Förde (*N. spumigena*) anwesend waren, konnte die trophische Anreicherung im Zooplankton  $\delta^{15}\text{N}$  nur im Hopavågen-Experiment getestet werden. Basierend auf ein Modell, wurde das Maß der Carnivorie eines Zooplankters vom Ausmaß der Steigung  $k$  einer Regressionsgeraden abgeleitet, die an die Zooplankton  $\delta^{15}\text{N}$  als Funktion von  $\text{Log}_{10}$ -transformierten Zooplanktondichten angepasst wurde. Diese Analyse fußt auf der Annahme, dass die trophische Anreicherung sich einerseits über den Anfangswert (also mit der Zeit) erhöhen wird, und zusätzlich mit abnehmender Copepoden-Dichte (bzw. zunehmender Futterdichte). Im Copepod-Treatment des Hopavågen-Versuchs, zeigten die Copepoden *Centropages hamatus* und *Pseudocalanus elongatus* die erwartete Zunahme des  $\delta^{15}\text{N}$  entlang des Copepoden-Gradienten ( $k = -0.91$  bzw.  $-0.62$ ). Der Copepode *Temora longicornis*, war dagegen in allen Säcken, unabhängig von der Tierdichte, in seinem  $\delta^{15}\text{N}$  im gleichen Ausmaß ( $\sim 0.4\text{‰}$ ) angereichert (daher,  $k = 0$ ). Dies lässt vermuten, dass *C. hamatus* in seiner Kost karnivorer war als *T. longicornis*, was mit den unterschiedlichen 'foraging modes' dieser Arten übereinstimmt ('cruising' versus 'stationary suspension feeding'). Jedoch ergeben sich Schwierigkeiten in der Interpretation von Zooplankton  $\delta^{15}\text{N}$  bei unterschiedlichem artspezifischen Nahrungsstress. Im Kieler Förde-Experiment führte die Entwicklung von *N. spumigena* zu einer signifikanten Abnahme des Seston  $\delta^{15}\text{N}$  (bis zu  $2.5\text{‰}$ ). Die Abnahme der Zooplankton  $\delta^{15}\text{N}$  mit der Zeit (ebenfalls bis zu  $2.5\text{‰}$ ), als auch deren

Korrelation mit dem Zooplankton-Gradienten, lässt den Schluss zu, dass diazotropher N in die Zooplankter gelangte, wobei der Großteil wahrscheinlich eher über einen indirekten, mikrobiellen Pfad als über direkte Ingestion von *N. spumigena* inkorporiert wurde. Die ähnliche Abnahme der Zooplankton  $\delta^{15}\text{N}$  mit der Copepoden-Dichte im Schöhsee-Experiment dürfte ebenfalls über einen solchen Transfer von diazotrophem N zu erklären sein. Die  $\delta^{15}\text{N}$  von *Daphnia* und calanoiden Copepoden zeigten, dass das herbivore Süßwasser-Zooplankton bis zu etwa einer trophischen Stufe von einander getrennt sein kann (3.2 bis 4.8‰ Differenz). Dagegen sind die Unterschiede zwischen dem herbivoren marinen Zooplankton – sogar zwischen *O. dioica* und den Copepoden – nur unwesentlich, mitunter nichtig. Bezüglich der zweiten Hypothese, kann also die Schlussfolgerung gezogen werden, dass die Interpretation von Zooplankton  $\delta^{15}\text{N}$  schwierig ist, weil die trophische Anreicherung nur einen von vielen Faktoren darstellt (z.B. Nahrungsstress, Aufnahme von diazotrophem N, Nitrat-Auftrieb, etc...), die zur Veränderung von Zooplankton  $\delta^{15}\text{N}$  führt.

Der Einfluss von Süßwasser-Copepoden auf die Stöchiometrie von Seston konnte nicht getestet werden, weil die Copepoden-Treatments zu Ende des Versuchs stark durch heranwachsende Cladoceren kontaminiert waren. In den *Daphnia*-Treatments nahmen die Seston C:P-Verhältnisse signifikant mit der Tierdichte zu. Dies war das Ergebnis einer relativ stärkeren Abnahme der P als der C-Konzentrationen im Seston. Berechnungen zeigten, dass der Verlust von P im Seston quantitativ der Menge an P, gebunden in ‚neuer‘ *Daphnia*-Biomasse (numerische Zunahme), entsprach. Somit ist die Zunahme der Seston C:P-Verhältnisse im Einklang mit den theoretischen Vorhersagen, weil P-reiche Cladoceren das ‚Mangel-Element‘ P in neuer Biomasse banden, und damit P-Limitation im Phytoplankton verursachten. Im Hopavågen-Experiment hatte die Zugabe der Copepoden zu den Mesokosmen zur Folge, dass das Seston proportional zur hinzugefügten Wassermenge mit N angereichert wurde. Das war wahrscheinlich ein unerwünschter Düngungseffekt mit Ammonium aus der Tonne, in dem die Tiere zuvor konzentriert worden waren. Dies, aber auch Experimente mit Nährstoff-Zugabe, zeigten, dass das Wachstum von Phytoplankton N-limitiert war. Wegen der anfänglich angereicherten C:N-Verhältnisse im Seston, wurde die Differenz zwischen dem Anfangs- und Endwert der C:N-Verhältnisse  $[\Delta(\text{C:N})_t]$  berechnet. Die  $\Delta(\text{C:N})_t$ -Verhältnisse des Sestons nahmen signifikant mit der Copepoden-Dichte zu. Der Grund war die relativ stärkere Zunahme der C im Vergleich zu den N-Konzentrationen im Seston. Letztere können der generell hohen Copepoden-Mortalität (und anschließender N-Abgabe aus den toten Tieren) zugeschrieben werden, da ja keine diazotrophen Blaualgen vorhanden waren. Die Zunahme der Seston  $\Delta(\text{C:N})_t$ -Verhältnisse entspricht jedoch nicht den theoretischen Vorhersagen, weil N nicht in tierischer Biomasse gebunden, sondern - im Gegenteil - von (toten) Copepoden abgegeben wurde. Der zugrundeliegende Mechanismus scheint die Veränderung der Phytoplankton-Größenverteilung zugunsten der Nanoflagellaten über eine trophische Kaskade ‘Copepoden-Ciliaten-Nanoflagellaten’ zu sein. Der starke Zuwachs der Nanoflagellaten zu Lasten des intrazellulären N-Pools verursachte schließlich die Zunahme der  $\Delta(\text{C:N})_t$ -Verhältnisse im Seston. Im Experiment in der Kieler Förde nahmen sowohl die C:N als auch

die C:P-Verhältnisse im Seston mit zunehmender Copepoden-Dichte ab. Dies geschah wegen relativ stärkerer Abnahmen der C als der N oder P-Konzentrationen im Seston. Da man annehmen kann, dass das Phytoplankton zu Anfang des Experiments nicht nährstoff-limitiert war [relativ ausgeglichene N:P-Verhältnisse ( $N:P = \sim 15$  to  $20$ ), hohe Konzentrationen von gelöstem anorganischem P und Ammonium], erscheint es sinnvoll, die Abnahmen der Seston C:N und C:P-Verhältnisse als das Resultat einer erhöhten Reduktion des Detritus-Anteils - den ja Copepoden fressen können - innerhalb der C-Fraktion zu sehen. Dieses Ergebnis ist im Einklang mit den Vorhersagen der ökologischen Stöchiometrie, weil ähnliche N:P-Verhältnisse von Herbivore und seiner Ressource - wie sie hier gewiss vorlagen - und der ‚kompensatorische‘ Zufluss von Nährstoffen (vom anorganischen P-Pool, und von diazotropher N-Fixierung) eine Nährstoff-Limitation des Phytoplanktons verhindern, selbst wenn die Copepoden-Populationen anwachsen.

Man kann daher den Schluss ziehen, dass weder in nährstoffreichen (Kieler Förde) noch nährstoffarmen (Hopavågen) marinen Systemen, das Konzept einer von Konsumenten getriebenen Nährstoff-Rezyklierung eine große Bedeutung hat. Süßwasser-Daphnien in Seen sind einfach anders als Copepoden im Meer: Sie sind effiziente Filtrierer, stöchiometrisch stark abweichend von ihrem Futter, und groß. Und, es ist dieser Unterschied, der zählt.

## Abstract

Herbivorous mesozooplankton are the major trophic link between phytoplankton and fish in both, marine and freshwater systems. They differ strongly in their body stoichiometry, and in the way they acquire food. Copepods grasp particles and may actively select for both, large phytoplankton and microzooplankton. They are relatively rich in nitrogen and, thus, have high body nitrogen to phosphorus (N:P) ratios. In contrast, mechanical sievers (e.g. freshwater cladocerans, appendicularians) are restricted to the filtration of generally smaller particles. They are relatively rich in P and, hence, have low body N:P ratios. Based on these findings, the following hypotheses were made: (1) Copepods shift the size structure of phytoplankton assemblages to small particles by removing both, large phytoplankton and microzooplankton. In contrast, mechanical sievers filter smaller particles and, thus, favour large phytoplankton. (2) Consequently, the nitrogen stable isotope signatures ( $\delta^{15}\text{N}$ ) of copepods – as an indicator of trophic enrichment – are higher than those of mechanical sievers, because microzooplankton contribute proportionately more to the diets of copepods. (3) In accordance with the theory of ecological stoichiometry, copepods with high body N:P ratios drive phytoplankton towards N-limitation, whereas zooplankton with low body N:P ratios cause phytoplankton P-limitation. This is because zooplankton preferentially retain the element in demand and recycle the element in excess to maintain stoichiometric balance.

These hypotheses were tested in 3 mesocosm studies during summer conditions; in (1) the freshwater lake Schöhsee, Northern Germany, in August 2000, in (2) the marine Hopavågen lagoon, Norway, in July 2001, and (3) in Kiel Fjord, Western Baltic, in September 2003. At all study sites, a copepod treatment of logarithmically scaled densities (5 to 80 or 160 ind  $\text{L}^{-1}$ ) was established using natural copepod assemblages. In the Schöhsee experiment, similarly, a density gradient was established with the laboratory-reared cladoceran *Daphnia hyalina x galeata* (2.5 to 40 ind  $\text{L}^{-1}$ ). At both marine sites, appendicularian treatments consisted of a set of bags, in which copepods were removed. This was done, because copepods may feed on juvenile appendicularians and/or their eggs. Indeed, the appendicularian *Oikopleura dioica* developed in the Hopavågen (max. 35 ind  $\text{L}^{-1}$ ), yet not in the Kiel Fjord experiment. The mesocosms (~1.5 or ~3.4  $\text{m}^3$  volume) were sampled every 3 to 4 days for counts of phytoplankton and zooplankton densities, and for carbon (C), N and P concentrations of pre-screened (<64 or <100  $\mu\text{m}$ ) particulate organic matter, here termed 'seston'.  $\delta^{15}\text{N}$  samples of zooplankton and seston were taken at the beginning and the end of the marine experiments. In the Schöhsee experiment, only final zooplankton  $\delta^{15}\text{N}$  samples were taken.

The Schöhsee phytoplankton community was diverse. Copepods and cladocerans had a negative impact on large (>4000  $\mu\text{m}^3$ ) and small (<4000  $\mu\text{m}^3$ ) phytoplankton, respectively. They were, hence, complementary in their impact on phytoplankton size. The Hopavågen food web was dominated by heterotrophic ciliates. Copepods equally had a negative impact on large particles (diatoms, ciliates), and positively affected small (<5  $\mu\text{m}$ ) nanoflagellates, presumably via a trophic cascade. No impact



was found for appendicularians. In the Kiel Fjord experiment, phytoplankton was also diverse. However, in contrast to the previous experiments, copepods affected similarly sized phytoplankton (diatoms) both, negatively and positively. Notably, the diazotrophic cyanobacterium *Nodularia spumigena* increased in all mesocosms. While a negative impact on cell concentrations was found on a short-term (3 days), no effect on *N. spumigena* cell concentrations or size frequency distributions of trichomes (indicative of ‘filament clipping’) were apparent in the long run (9 days). With respect to the first hypothesis, it is concluded that freshwater copepods and cladocerans may indeed shift phytoplankton size structure to small and large species, respectively. In marine oligotrophic systems (Hopavågen), copepods may also select phytoplankton on the basis of particle size, but perhaps become more selective towards the ‘taste’ of phytoplankton (diatoms) under meso to eutrophic conditions (Kiel Fjord).

Due to the presence of diazotrophic cyanobacteria in the Schöhsee (*Anabaena flos-aquae*, *Microcystis* sp.) and Kiel Fjord experiments (*N. spumigena*), trophic enrichment of the zooplankton  $\delta^{15}\text{N}$  could be tested only in the Hopavågen experiment. According to a model, the extent of carnivory of a zooplankton was predicted from the slope  $k$  of a linear regression fitted to the zooplankton  $\delta^{15}\text{N}$  as a function of  $\text{Log}_{10}$ -transformed copepod densities. This was based on the assumption that trophic enrichment would increase with respect to start  $\delta^{15}\text{N}$  (hence, with time), and as zooplankton densities decreased (and therefore food availability increased). In the Hopavågen copepod treatment, the copepods *Centropages hamatus* and *Pseudocalanus elongatus* showed the expected increases along the density gradient ( $k = -0.91$  and  $-0.62$ , respectively). In contrast, the copepod *Temora longicornis* was equally enriched by  $\sim 0.4\text{‰}$  in all treatments (therefore,  $k = 0$ ), irrespective of copepod density. This suggests that *C. hamatus* was more carnivorous than *T. longicornis*, which is consistent with their differing foraging modes (cruising versus stationary suspension feeding). Difficulties in the interpretation of zooplankton  $\delta^{15}\text{N}$  may arise with species-specific isotopic enrichment due to nutritional stress. In the Kiel Fjord experiment, the development of *N. spumigena* significantly decreased the  $\delta^{15}\text{N}$  of seston by up to  $2.5\text{‰}$ . Decreases of zooplankton  $\delta^{15}\text{N}$  with time (equally up to  $2.5\text{‰}$ ), and along the zooplankton density gradient suggest that diazotrophic N entered zooplankton mainly via indirect, microbial pathways, rather than by direct ingestion of *N. spumigena*. Equally, the transfer of diazotrophic N may explain the decreases of zooplankton  $\delta^{15}\text{N}$  with copepod abundances seen in the Schöhsee experiment. The  $\delta^{15}\text{N}$  of *Daphnia* and calanoid copepods suggest that herbivorous freshwater zooplankton may be as far as one-trophic level apart ( $3.2$  to  $4.8\text{‰}$  difference). In contrast, differences between marine zooplankton – also between *O. dioica* and copepods – seem negligibly small. With respect to the second hypothesis, it may be concluded that zooplankton  $\delta^{15}\text{N}$  are difficult to interpret, because trophic enrichment is only one of many factors (e.g. nutritional stress, incorporation of diazotrophic N, nitrate upwelling, etc...) that influence changes in zooplankton  $\delta^{15}\text{N}$ . The stoichiometric impact of freshwater copepods could not be tested, because the copepod treatments became strongly contaminated with cladocerans towards the end of the experiment. In the *Daphnia*

treatment, seston C:P ratios significantly increased with *Daphnia* densities. This was due to relatively stronger decreases of seston P than seston C concentrations. Calculations show that the drain of P from seston corresponded quantitatively to the amount of P bound in 'new' *Daphnia* tissue (numerical increase). Thus, the increase of seston C:P ratios is consistent with theoretical predictions, since P-rich *Daphnia* retained the element in demand (P) in new biomass, thereby driving phytoplankton towards P-limitation. In the Hopavågen experiment, the addition of copepods to the mesocosms enriched seston with N proportionately with the amount of added water. This was probably an undesired enrichment effect with ammonia from the barrel in which copepods were previously concentrated. This, and nutrient enrichment bioassays, indicated that phytoplankton growth was limited by N. Because of the initially enriched seston C:N ratios, the difference between start and final seston C:N ratios  $[\Delta(C:N)_t]$  was calculated. Seston  $\Delta(C:N)_t$  ratios significantly increased with copepod densities due to relatively stronger increases of seston C than seston N concentrations. The increases of N may be attributed to the generally high copepod mortalities (and subsequent release of N from dead copepods), since diazotrophic algae were absent. The increase of seston  $\Delta(C:N)_t$  ratios is, however, not consistent with theoretical predictions, because N was not retained, but released from (dead) copepods. Instead, the responsible mechanism seemed to be the shift in the size structure of phytoplankton to nanoflagellates mediated via a trophic cascade 'copepods-ciliates-nanoflagellates'. Thus, increased nanoflagellate growth at the expense of the intracellular N pool resulted in increased seston  $\Delta(C:N)_t$  ratios. In the Kiel Fjord experiment, both, seston C:N and C:P ratios decreased with copepod abundances. This was due to relatively stronger decreases of seston C than seston N and P concentrations, respectively. Since phytoplankton was considered not to be nutrient limited at the onset of the experiment [relatively balanced N:P ratios (N:P= ~15 to 20), high concentrations of inorganic dissolved P and ammonia], the decreases of seston C:N and C:P ratios were rather interpreted as the result of increased reduction of detritus within the seston C fraction, which copepods may indeed feed on. This finding is consistent with the predictions of ecological stoichiometry, because similar grazer-resource N:P ratios – which were almost certainly the case here – and/or a 'compensatory' supply of nutrients (from the inorganic P pool, and from diazotrophic N fixation) prevent nutrient limitation of phytoplankton, even if copepod populations increase.

It may, therefore, be concluded that for both, nutrient-rich (Kiel Fjord) and nutrient-poor (Hopavågen) marine systems, the concept of consumer-driven nutrient recycling is not of great importance. Freshwater *Daphnia* in lakes, are simply different from copepods in the sea: They are effective grazers, stoichiometrically very different from their food, and big. And, it is this difference that matters.

## I. General introduction

In aquatic systems, mesozooplankton – hereafter referred to as zooplankton – are the major trophic link between phytoplankton and fish (Cushing 1975, Carpenter et al. 1985). In the classical view of pelagic food chains, zooplankton are assumed to feed mainly on phytoplankton. Yet, in the past decades it has become acknowledged that most herbivorous zooplankton are omnivorous to some degree, depending on their feeding strategy (Greene 1988), prey size and motility (Berggreen et al. 1988, Tiselius and Jonsson 1990), turbulence (Saiz and Kjørboe 1995) and food web structure (Ohman and Runge 1994). Additionally, phytoplankton might not always be nutritionally adequate, which may strongly influence zooplankton performance (Sterner 1993, Urabe et al. 1997, Jónasdóttir et al. 1998) and selectivity (Cowles et al. 1988, Butler et al. 1989). Nevertheless, zooplankton abundance generally follows spatial (up-welling regions) and temporal (spring blooms) patterns of global primary productivity, and recent evidence (Irigoin et al. 2002) emphasises the importance of the ‘classical’ direct link between phytoplankton and zooplankton production.

In aquatic systems, both zooplankton and phytoplankton experience similar ecological constraints, especially those imposed by their fluid environment. Therefore, it is not surprising that marine and freshwater plankton are to a great extent composed of closely related taxa. Some important groups, though, are absent in freshwater and occur only in marine environments (e.g. silicoflagellates, appendicularians, chaetognaths). Although herbivorous zooplankton occupy similar functional positions in aquatic food webs, they show marked differences in two important aspects:

- (1) Major taxa and even species with a taxon differ in the way they acquire food. Mechanical retention by means of sieving devices in cladocerans, pelagic tunicates, and meroplanktonic larvae (Strathman and Leise 1979, Geller and Müller 1981, Flood et al. 1992) opposes remote particle perception, ‘tasting’ and selective ingestion by calanoid copepods (Bundy et al. 1998, DeMott 1988). Also, continuous swimming by cruising copepods and cladocerans contrasts with stationary suspension feeding by other calanoid copepods (Greene 1988). Such differences in prey acquisition, and the range of ingested particle sizes this implies, have been proposed to structure pelagic food webs (Jürgens 1994, Sommer and Stibor 2002).
- (2) There are marked differences in population dynamics. Copepods reproduce sexually and show extended ontogenetic shifts from larval (nauplii) to subadult and adult stages. Generation times are therefore longer for copepods than for parthenogenetically reproducing cladocerans or fast growing pelagic tunicates. This has implications for the speed with which populations of different zooplankton may respond to increases in prey abundance, but also for zooplankton stoichiometry. Enhanced reproduction results in lower nitrogen to phosphorus (N:P) body ratios of zooplankton, because most P is bound in ribosomal RNA necessary for growth (Main et al. 1997). Such differences in zooplankton stoichiometry may affect patterns of specific (N or P) nutrient limitation of phytoplankton growth (Elser et al. 1988), within and beyond (marine and freshwater) systems (Elser and Hassett 1994).

In my thesis, I have attempted to find similarities and differences in the impact of major zooplankton taxa on natural summer phytoplankton assemblages. This was studied in mesocosm experiments in three different aquatic systems: a freshwater lake (Schöhsee), a marine oligotrophic bay (Hopavågen, Norway) and a brackish Baltic fjord (Kiel Fjord). Marine and freshwater species of three major zooplankton taxa were used (copepods, cladocerans, appendicularians) and their impact analysed with respect to (1) shifts in the size structure of phytoplankton assemblages, and (2) the relative nutrient content of phytoplankton cells. Additionally, nitrogen stable isotope measurements (3) were performed in order to reveal potential differences in the degree of zooplankton herbivory.

I have arranged my thesis in chapters according to major aspects (particle grazing, stable isotopes, stoichiometry), rather than presenting all data of each mesocosm experiment together. At the beginning of each chapter a short introduction is given, which includes the problem and the expected outcome of the experiments. The results within each chapter are generally presented in a chronological order, hence starting with the freshwater experiment, followed by the marine experiments in Norway and Kiel Fjord. This was changed in Chapter IV for reasons of clarity. The results of each experiment are discussed together with the results (Chapter III) or, separately, thereafter (Chapters IV and V). The findings of all three experiments are summarised, and conclusions are drawn at the end of each chapter.

## II. Materials and Methods

### II.1 Study sites

The mesocosm experiments were conducted at three different sites between August 2000 and September 2003: in (1) a mesotrophic lake ('Schöhsee'), Plön, Northern Germany, (2) a sheltered, semi-enclosed marine lagoon ('Hopavågen'), situated on the outlet of the Trondheim Fjord, Norway, and (3) in Kiel Fjord, Western Baltic Sea (Table 1). Although all mesocosm experiments were conducted during the temperate summer, physical conditions varied widely. The Schöhsee and Kiel Fjord experiments showed greatest similarities with relatively high water temperatures (~20°C) and high solar radiation due to pleasant weather conditions. Moreover, both sites were characterised by a highly diverse phytoplankton community, which was in contrast to the 'microbial loop' dominated Hopavågen food web (see Chapter III.3). However, chl a content did not reflect differences in food web structure (high versus low phytoplankton dominance), but instead indicated greater similarities for the marine sites. It is important to note that during the first experiment in Schöhsee, calcite precipitated visibly in both the lake and the mesocosm bags, a frequent phenomenon in lakes termed 'whiting'. This event had a great impact on P availability (see Chapter V.2), as phosphate easily co-precipitates with calcite (Kleiner 1988) and thus makes P unavailable to phytoplankton.

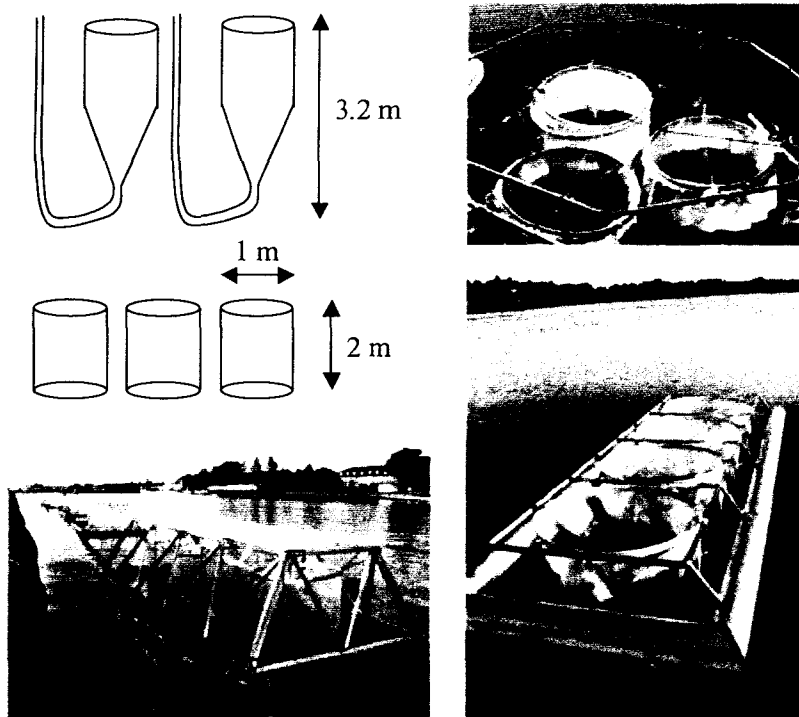
**Table 1:** General water parameters and weather conditions at the three study sites during the experimental periods. Values are ranges.

study site	period	water temp. [°C]	salinity [PSU]	chl a [µg L <sup>-1</sup> ]	weather conditions
Schöhsee Plön, GERMANY	9-28 August 2000	18.6 - 20.2	0	1.6 - 3.2	dry & sunny
Hopavågen Trondheim, NORWAY	16-22 July 2001	12.5 - 13.3	30.8 - 31.1	0.08 - 1.8	cloudy & rainy
Kiel Fjord Kiel, GERMANY	4-13 September 2002	19.4 - 20.0	11.8 - 14.3	0.4 - 1.7	dry & sunny

### II.2 Experimental design

**Mesocosms** - The mesocosms consisted of 24 transparent polyethylene bags (Trikoron, BP chemicals), suspended in several floats (Figure 1). All enclosure bags were 1 m in diameter and open to the top. In the first experiment (Schöhsee), the top 2 m of the mesocosm bags was cylindrical, whereas the bottom part was conically shaped and ended in a tube used to remove accumulated

particulate matter ('sediment'). In this experiment, the bags were ~3.2 m deep, and comprised ~3.4 m<sup>3</sup> lake water. As zooplankton tended to accumulate within the lower part of the bags, at times many zooplankton were removed with the sediment. Therefore, in the following marine experiments, bags were simply sealed at the bottom, where weights were attached to keep the bags suspended upright. The resulting shape was approximately cylindrical, ~2.5 m deep and contained ~1.5 m<sup>3</sup> seawater.



**Figure 1:** The mesocosm bags. Simplified scheme of the bags used in the freshwater and marine experiments (top left). Pictures of the mesocosm arrangements at the Hopavågen, Norway, in July 2001 (top right), at the WSA, Kiel-Holtenau, in September 2002 (bottom left) and in the Schöhsee, Plön, in August 2000 (bottom right). In the Hopavågen and Kiel Fjord experiments, the bags were covered with transparent foil mounted in frames to protect bag contents from bird faeces.

The mesocosm bags were filled with lake water (50 µm pre-screened) pumped from 1 m depth (Schöhsee), or by hauling the submerged bags from ~3 m depth to the surface (marine experiments). Therefore, zooplankton that had entered the bags during the filling procedure in the marine experiments, had to be removed from within the bags by means of several net hauls (250 µm mesh size, 0.8 m diameter). In the freshwater experiment, mesocosm bags were fertilised with inorganic phosphate (106 mg P bag<sup>-1</sup>) after filling them in order to obtain a more balanced nutrient ratio closer to Redfield (N:P = 16:1). This was based on measurements of total nitrogen (TN) and total phosphorus (TP) prior to fertilisation indicating P limitation (molar TN:TP = 54:1). In the marine experiments,

where N limitation was expected, fertilisation was not performed in order to conserve natural zooplankton  $\delta^{15}\text{N}$  signatures.

**Treatments** - The intention in all mesocosm experiments was to compare the impact of at least two dominant zooplankton taxa differing in their feeding ecology. In the Schöhsee experiment, a cladoceran and a copepod treatment were established (Table 2). In both marine experiments, a copepod treatment was compared to a set of bags in which zooplankton were initially removed and expectantly termed ‘appendicularian treatment’. This was done because the removal of copepods in a previous mesocosm experiment in the Hopavågen had resulted in the development of appendicularians (Stibor et al. 2003). This procedure, in fact, proved again successful in the Hopavågen experiment (see appendix, Sommer et al. 2003b), but not in the Kiel Fjord experiment.

(I need to note that several additional treatments to those given in Table 2 were established during the stay in Norway, and also in the Baltic mesocosm experiment. For reasons of clarity, I have decided to concentrate only on the ‘main’ treatments in my thesis, and accordingly data of other treatments are not shown.)

**Table 2:** Treatments and dominant zooplankton species at the three study locations. ‡ denotes that zooplankton  $>250\ \mu\text{m}$  was removed in order to induce population growth of appendicularians.

study site	treatment	species	no. bags	seeding densities [ ind L <sup>-1</sup> ]
Schöhsee	cladoceran	<i>Daphnia hyalina</i> x <i>galeata</i>	11	1.25, 2.5, 5, 10, 20, 40
	copepod	<i>Eudiaptomus gracilis</i> cyclopoid copepods	11	5, 10, 20, 40, 80, 160
Hopavågen	appendicularian copepod	<i>Oikopleura dioica</i>	6	‡
		<i>Temora longicornis</i>	10	5, 10, 20, 40, 80
		<i>Centropages hamatus</i>		
		<i>Centropages typicus</i>		
		<i>Pseudocalanus elongatus</i>		
Kiel Fjord	appendicularian	-	6	‡
	copepod	<i>Acartia clausi</i>	10	5, 10, 20, 40, 80

Each treatment (except for the appendicularian) consisted of a gradient of zooplankton densities. Treatment densities were scaled logarithmically (e.g. 5, 10, 20, 40, 80 ind L<sup>-1</sup>, Table 2). The density gradient was established by adding an increasing amount of zooplankton to the mesocosm bags. Yet, since water volumes in the mesocosm bags were only approximate, actual initial zooplankton densities differed in some cases from intended (seeding) densities. Highest zooplankton treatment densities exceeded naturally occurring maximum abundances, maximally two-fold. Each treatment density, except for the lowest in the Schöhsee experiment, was replicated twice. In all experiments, 2 bags

receiving no zooplankton served as control treatments. In control bags of the Hopavågen mesocosm, zooplankton recruiting from nauplii or early copepodite stages were daily removed by means of 10 vertical net hauls (250 µm mesh size). In the Schöhsee and Baltic experiments, this procedure was not performed due to the abundance of large phytoplankton which would have been partially removed together with the zooplankton. In the appendicularian treatment of the Hopavågen experiment, appendicularians developed 9 d after filling the bags resulting in a natural density gradient of 0 to 35 ind L<sup>-1</sup> (see appendix).

**Zooplankton** - In the Schöhsee experiment, bags of the cladoceran treatment were inoculated with laboratory-reared *Daphnia hyalina* x *galeata* obtained from the Max-Planck-Institute of Limnology in Plön (Table 2). Copepods were collected with a plankton net (250 µm mesh size) in the lake, and consisted mainly of the calanoid species *Eudiaptomus gracilis* (or possibly the closely related *E. graciloides*), and of few copepodite stages of cyclopoid copepods. The contents of the net hauls (copepods and co-occurring cladocerans) were first concentrated in barrels (300 L volume) and submitted to heavy bubbling with air for 7 h, before adding copepods to the copepod treatment. Although this procedure effectively removed adult cladocerans, contamination by surviving, rapidly reproducing cladocerans in the copepod treatment was evident along the course of the experiment. The numerical scaling of copepods in the copepod treatment was four-fold that of *Daphnia* in the cladoceran treatment. This was done in order to achieve a comparable zooplankton biomass gradient, based on the assumption that *Daphnia* biomass (~17 µg dry weight ind<sup>-1</sup> for *D. hyalina*, Santer 1990) was approximately four times that of the calanoid *Eudiaptomus* [4 µg dry weight ind<sup>-1</sup> for *Eudiaptomus*, calculated from (1978) and Bottrell et al. (1976)]. The highest seeding densities in the freshwater experiment (40 and 160 ind L<sup>-1</sup>, respectively) exceeded naturally occurring maximum abundances of *Daphnia* sp. and *Eudiaptomus* sp. in the lake by a factor of up to 2 (Fußmann 1996).

In both marine experiments, zooplankton was collected by means of horizontal tows with a plankton net (250 µm mesh size). In Norway, towing was performed at ~2 m depth within the Hopavågen lagoon, whereas Baltic zooplankton was collected at ~4 m depth at a deep (~15 m) site close to Laboe in Kiel Fjord. The Hopavågen zooplankton consisted of a mixed copepod assemblage dominated by the calanoids *Temora longicornis*, *Centropages hamatus*, *Centropages typicus* and *Pseudocalanus elongatus*. In contrast, zooplankton collected from Kiel Bight was almost entirely composed of the calanoid copepod *Acartia clausi* (the copepod *C. hamatus* and cladocerans were also present, albeit at low numbers, see Chapter IV.5). The maximum seeding density of copepods (80 ind L<sup>-1</sup>) represented ~3 times their abundance maximum in the Hopavågen in July (N. Tokle, unpublished data), but was only slightly higher than the maximum *in situ* values during the experimental period (~60 ind L<sup>-1</sup>). In Kiel Bight, long-term abundances of calanoid copepods are generally ~10 ind L<sup>-1</sup> in summer (Behrends 1997), yet abundances of calanoids may at time exceed 60 ind L<sup>-1</sup> at this time of the year (G Behrends, unpublished data).



As in the Schöhsee experiment, contents of zooplankton tows were first collected in barrels (300 L). Aeration was performed in Norway due to the (stronger) presence of the cladoceran *Evadne*, but not in the Baltic mesocosm experiment. Dead and injured individuals were allowed to sink to the bottom of the barrels, from where they were removed prior to their addition to the mesocosm bags. The addition of zooplankton from barrels, in which they had been previously concentrated, had an impact on nutrient concentrations in the Hopavågen mesocosm. Concentrations of ammonia and phosphate released from zooplankton within the barrels were fairly high:  $\sim 13 \mu\text{mol NH}_4 \text{ L}^{-1}$  and  $1.1 \text{ PO}_4 \mu\text{mol L}^{-1}$  (data from a second mesocosm study in April 2002 in the Hopavågen). As appropriate amounts of the 'barrel water' was added to the mesocosm bags together with the zooplankton, the seston became increasingly enriched with N as zooplankton densities increased (see Chapter V.3). In the Schöhsee experiment, this effect was avoided by adding appropriate amounts of the barrel water without zooplankton, complementary to the highest amount of water added in the highest density treatments. On all three occasions, zooplankton collection was characterised by the fortunate circumstance that  $>250 \mu\text{m}$  net plankton consisted exclusively of zooplankton. Thus, addition of zooplankton to the bags did not result in contamination of the bags with large algae within the same size range as zooplankton (e.g. large *Ceratium* species or chain-forming diatoms).

### II.3 Variables and calculations

All bags were sampled for plankton counts and nutrient chemistry on a 3 to 4 day-interval. Prior to sampling, the entire enclosed water body was mixed with a Secci disc. A list of the most important variables measured in each mesocosm experiment is given in Table 3.

Chl a concentrations were determined in both, the Schöhsee and the Hopavågen experiment, using a fully submersible bbe Fluoroprobe (bbe Moldaenke), which directly measures chl a content *in situ* and *in vivo* (Beutler et al. 2002). In the Baltic experiment, chl a concentrations were measured *in vivo* using a Turner Designs fluorometer. Temperature and salinity were measured synchronously using a conductivity meter (LF 320, Tetracon).

Samples for analytical analyses (but not Utermöhl counts) were pre-screened in order to remove zooplankton. In the Schöhsee and Hopavågen experiments, samples were  $100 \mu\text{m}$  pre-screened. In the Kiel Fjord experiment, samples were  $64 \mu\text{m}$  pre-screened in order to exclude copepod eggs ( $\sim 80 \mu\text{m}$ ) from the filters, as especially high fecundity was expected.

Samples ( $\sim 10 \text{ ml}$ ) for the analysis of inorganic dissolved nutrients ( $\text{PO}_4$ ,  $\text{NO}_3 + \text{NO}_2$ ,  $\text{NH}_4$  and  $\text{SiO}_4$ ), as well as total nitrogen (TN) and total phosphorous (TP) were measured immediately after sampling in an autoanalyser (Skalar SANplus). TN and TP are defined here as excluding zooplankton. Samples for the analysis of seston C, seston N and seston P content were filtered onto precombusted ( $550^\circ\text{C}$ , 24 h), acid-washed (10% HCl) Whatmann GF/F filters. After drying ( $\sim 24 \text{ h}$ ), samples for seston C and N analysis were stored in a desiccator until combustion in a CHN-analyser (Fisons, 1500N). Samples for particulate P analysis were measured as orthophosphate according to Grasshoff et al. (1999) after

oxidative digestion. The term ‘seston’ is used throughout the text, meaning particulate organic matter (POM) excluding zooplankton. It was measured as a surrogate for zooplankton diet. Technically, it though also includes detritus. The terms seston C, N and P are accordingly used instead of POC, PON and POP. In keeping with the tradition of limnologists and marine scientists, units of elemental contents per unit volume are given in  $\mu\text{g L}^{-1}$  or  $\mu\text{mol L}^{-1}$ , respectively.

**Table 3:** The main variables measured in the mesocosm studies. Pre-screening was  $<100\ \mu\text{m}$  in the Schöhsee and Hopavågen, and  $<64\ \mu\text{m}$  in the Kiel Fjord experiments. Units of elemental concentrations were given in  $\mu\text{g L}^{-1}$  (Schöhsee) or  $\mu\text{mol L}^{-1}$  (Hopavågen, Kiel Fjord), respectively. Note that in the following chapters not all data are shown for each mesocosm study.

Variables	screening limits		units
	lower	upper	
Chl a content	-	-	$\mu\text{g L}^{-1}$
Temperature	-	-	$^{\circ}\text{C}$
Salinity	-	-	PSU
Seston (=POM)			
Seston C, N, P (=POC, PON, POP)	GF/F	$<100 / <64\ \mu\text{m}$	$\mu\text{g L}^{-1} / \mu\text{mol L}^{-1}$
Seston C:N, C:P, N:P	GF/F	$<100 / <64\ \mu\text{m}$	-
TN, TP	-	$<100 / <64\ \mu\text{m}$	$\mu\text{g L}^{-1} / \mu\text{mol L}^{-1}$
Dissolved inorganic nutrients			
NO <sub>3</sub> , NH <sub>4</sub> (=DIN)	-	$<100 / <64\ \mu\text{m}$	$\mu\text{g L}^{-1} / \mu\text{mol L}^{-1}$
SRP / PO <sub>4</sub> (=DIP)	-	$<100 / <64\ \mu\text{m}$	$\mu\text{g L}^{-1} / \mu\text{mol L}^{-2}$
SiO <sub>4</sub>	-	$<100 / <64\ \mu\text{m}$	$\mu\text{g L}^{-1} / \mu\text{mol L}^{-3}$
Sediment C, N, P	GF/F	-	$\mu\text{g L}^{-1}$
Plankton counts (Utermöhl)	-	-	cells $\text{mL}^{-1}$
Zooplankton counts	-	-	ind $\text{L}^{-1}$
Seston $\delta^{15}\text{N}$	GF/F	$<100 / <64\ \mu\text{m}$	‰
Zooplankton $\delta^{15}\text{N}$	-	-	‰

Sediment samples (only in the Schöhsee experiment) were obtained by removing the bottom water layer ( $\sim 5\ \text{L}$ ) by means of a hand-pump via a tube attached to the conically shaped bottom of the mesocosm bags. Subsamples (50 ml) were filtered onto pre-combusted and acid-washed Whatmann GF/F filters and analysed as described for seston samples.

Unfiltered samples for counts of phytoplankton were preserved with acid Lugol’s solution. Cell densities were determined under an inverted microscope (Leica DM IRB) following Utermöhl (1958). Chambers of 10, 30, 50 or 100 ml chambers were used, depending on the size and concentrations of phytoplankton. The size of large colonial algae (e.g. *Dinobryon*) was determined as ‘effective’ size (the size of the whole colony), not individual cell size. Colonies of *Nodularia spumigena* were

measured to the closest 10  $\mu\text{m}$  (trichomes were often  $>1000 \mu\text{m}$  in length), and trichome length (TL) converted to cell numbers (Cell N) using a conversion factor of

$$\text{TL:Cell N} = 0.28 \mu\text{m cell}^{-1}. \quad (1)$$

Specific phytoplankton growth rates ( $\mu$ ) were calculated as

$$\mu [\text{d}^{-1}] = \text{Ln} [(\text{cells ml}^{-1})_a / (\text{cells ml}^{-1})_b] / c, \quad (2)$$

where  $a$  is the final day considered,  $b$  is the start day, and  $c$  is the total number of days.

Biovolume ( $V$ ) was calculated assuming simple geometrical figures using length measurements (400x magnification,  $n = 10$  to 20) or class means. Diatom biomass ( $C$ ) was estimated from the relationship

$$C [\text{pg C}] = 0.11 \times \text{plasma volume} [\mu\text{m}^3], \quad (3)$$

where plasma volume was estimated from biovolume assuming a  $1 \mu\text{m}$  lining with  $F = 0.0$  according to Strathmann (1967). Nanoflagellate and ciliate biomass ( $C$ ) was calculated according to Putt and Stoecker (1989) using a conversion factor of

$$C:V = 0.19 \text{ pg C } \mu\text{m}^{-3}. \quad (4)$$

Otherwise, phytoplankton biomass ( $C$ ) was calculated from biovolume estimates according to Nalewajka (1966) using a conversion factor of

$$C:V = 0.1 \text{ pg C } \mu\text{m}^{-3}. \quad (5)$$

Relative changes of seston C and N content, and of seston C:N ratios with time [ $\Delta C_t$ ,  $\Delta N_t$ ,  $\Delta(\text{CN})_t$ ] were calculated as

$$\Delta X_t [\mu\text{mol L}^{-1}, \text{ if applicable}] = X_a - X_b, \quad (6)$$

where  $X$  is the element or ratio,  $a$  is the final day considered and  $b$  is the start day. The critical food threshold ratio  $Q^*_{F-e}$  (Urabe and Watanabe 1992) was calculated from:

$$Q^*_{F-e} = K_Z Q_{Z-e}, \quad (7)$$

where  $Q_{Z-e}$  is the zooplankton elemental ratio ( $e = \text{C:N}$  or  $\text{C:P}$ , respectively) and  $K_Z$  is the C gross growth efficiency of a zooplankton (assumed as 20-40%: Straile 1997). The stoichiometric imbalance  $\Delta(\text{N:P})_{\text{imb}}$  (Elser and Hassett 1994) was calculated from

$$\Delta(\text{N:P})_{\text{imb}} = \text{N:P}_F - \text{N:P}_Z, \quad (8)$$

where  $\text{N:P}_F$  and  $\text{N:P}_Z$  are the N:P ratios of food and zooplankton, respectively.

As the emphasis in the studies lay on the determination of trophic levels, generally only zooplankton  $\delta^{15}\text{N}$  are presented. In all cases carbon stable isotopes ( $\delta^{13}\text{C}$ ) were measured simultaneously and are shown in some figures. Start samples for the analysis of zooplankton  $\delta^{15}\text{N}$  were taken from barrels in which zooplankton tows were initially concentrated. No start samples were taken in the Schöhsee experiment. Final zooplankton samples were collected with a  $41 \mu\text{m}$  zooplankton net at the end of each experiment. All zooplankton samples were preserved in 90% ethanol. For each  $\delta^{15}\text{N}$  measurement, several individuals (only intact and preferably non-egg-carrying adults) were picked under a dissecting microscope and transferred onto pre-combusted GF/F filters (Schöhsee) or into tin cups (marine mesocosms). The number of individuals per sample ranged from 20 to 100 for all

zooplankton, but the appendicularian *O. dioica* (120 to 200). The  $\delta^{15}\text{N}$  of seston was determined from pre-screened (100 or 64  $\mu\text{m}$ ) samples filtered onto pre-combusted GF/F filters (200 to 1500 ml volume). After drying over night at 40°C, samples were stored in a desiccator until combustion in a CHN-analyser (Fisons, 1500N) connected to a Finnigan Delta Plus mass spectrometer.  $\delta^{15}\text{N}$  (and  $\delta^{13}\text{C}$ ) signatures were calculated as

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} [\text{‰}] = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000, \quad (9)$$

where  $R = (^{15}\text{N}/^{14}\text{N})$  or  $(^{13}\text{C}/^{12}\text{C})$ . Pure  $\text{N}_2$  and  $\text{CO}_2$  gas were used as a primary standard and calibrated against IAEA reference standards (N1, N2, N3, NBS22 and USGS24). A laboratory-internal standard (acetanilide) was measured after every fourth to sixth sample, encompassing a range of nitrogen comparable to the amount of zooplankton nitrogen. Samples were measured in several runs with a precision of  $\pm 0.2\text{‰}$  ( $\delta^{15}\text{N}$ ) and of  $\pm 0.1\text{‰}$  ( $\delta^{13}\text{C}$ ). Samples containing  $<15 \mu\text{g N}$  were excluded from further analyses.

Zooplankton was sampled by means of vertical hauls with a 50  $\mu\text{m}$  quantitative plankton net ( $\sim 12 \text{ L}$  volume) and preserved in 4% formalin final concentration. Subsamples of at least 200 individuals were counted to calculate zooplankton abundances. The absolute copepod growth rate ( $g$ ) was calculated as

$$g [\text{Ln} (\text{ind L}^{-1}) \times \text{d}^{-1}] = [\text{Ln} (\text{ind L}^{-1})_a - \text{Ln} (\text{ind L}^{-1})_b] / c, \quad (10)$$

where  $a$  is the final day considered,  $b$  is the start day, and  $c$  the total number of days. The relative change of copepod abundance with time ( $\Delta\text{Cop}_t$ ) was calculated as

$$\Delta\text{Cop}_t [\text{ind L}^{-1}] = \text{Cop}_a [\text{ind L}^{-1}] - \text{Cop}_b [\text{ind L}^{-1}], \quad (11)$$

where  $\text{Cop}_a$  and  $\text{Cop}_b$  are the copepod abundances on the final and start day, respectively.

## II.4 Data analysis

The impact of zooplankton was statistically analysed by fitting regressions to data of different variables (e.g. concentrations of phytoplankton species) plotted as a function of zooplankton abundance. Both, regression models and the choice of the ‘appropriate’ zooplankton density (time-averaged or ‘single-day’) varied.

For the analysis of zooplankton particle grazing in the Schöhsee and Hopavågen experiments, the regression model  $y = ax^b$  was used, where  $x$  is (mean) zooplankton abundance and the exponent  $b$  an integrated measure of the zooplankton impact on a phytoplankton species (or parameter). In the Schöhsee experiment, zooplankton seeding densities were used as zooplankton abundance (data published in: Sommer et al. 2001). In the Hopavågen mesocosm experiment, zooplankton mean abundance was calculated as the arithmetic mean of copepod densities determined for all sampling days between the initial and the final day of analysis. Such time-averaging should account for the fact that dietary particle concentrations are a time integrated response to zooplankton density during the

pre-sampling period. The exponent  $b$  includes the effects of direct grazing, grazing on intermediate consumers (microzooplankton) and nutrient regeneration. Hence, negative values of the exponent  $b$  result primarily from physical cell destruction or ingestion by zooplankton, whereas positive  $b$  values are mainly due to the release from grazing pressure by an intermediary taxon via a trophic cascade, and/or from growth enhancement by 'sloppy feeding', defaecation and excretion.

In the Kiel Fjord experiments, initial cell concentrations of most phytoplankton differed notably between bags. This may be attributed to the net hauls performed in order to remove zooplankton after filling the bags, a patchy distribution *per se* and/or vertical migration of phytoplankton (e.g. dinoflagellates: Kamykowski et al. 1998). In order to account for this initial patchy distribution, I used growth rates, rather than cell concentrations for statistical analysis. Furthermore, I considered a short-term and a long-term impact in order to account for time-dependent processes (e.g. food switching according to food availability), but also for the elimination of certain taxa by the end of the experiment. Growth rates were correlated with  $\text{Log}_{10}$ -transformed mean copepod abundances. Similar to the exponent  $b$ , the slope of a linear regression fitted to the data was interpreted as a positive or a negative impact according to the sign of the slope.

Nitrogen stable isotope signatures were analysed using linear regressions and  $\text{Log}_{10}$ -transformed mean (initial to final) copepod densities in order to account for time averaging.

Due to very different responses of elemental seston content or stoichiometric ratios to the addition and grazing of zooplankton, various regression models (linear, exponential, binomial second-order) were applied. Initially (Schöhsee experiment), I used actual, 'single-day' zooplankton abundances as abscissa data (data published in: Sommer et al. 2003a). This seems appropriate because it accounts for the transfer of elements in zooplankton tissues to the seston pool, when zooplankton die. Data points thus 'shift' to the left and to the top in a graph, when zooplankton population growth is negative. In the marine experiments, I used  $\text{Log}_{10}$ -transformed copepod densities for reasons of homogeneity of data.

Statistical values ( $r^2$ ,  $p$ ) were taken from regression analyses using SigmaPlot 8.0 and SigmaStat 2.03. Generally,  $p$  values were assigned as statistically significant at  $p < 0.05$ . The significance level was set higher ( $p = 0.1$ ) for correlations of phytoplankton, ciliate and rotifer growth rates with copepod mean densities, because growth rates were calculated from cell concentrations of two sampling days, which increases the error of quantification.

### III. Impact on phytoplankton abundances

#### III.1 Introduction

Herbivorous zooplankton differ in the way they collect food particles. Both, cladocerans and appendicularians mechanically sieve water by means of their filtering devices. While the fine mesh of appendicularians houses primarily retains small particles ( $<2$  to  $5\ \mu\text{m}$ : Flood et al. 1992, Sommer et al. 2000), freshwater cladocerans retain particles within a size range of  $\sim 1$  to  $30\ \mu\text{m}$  (Geller and Müller 1981). For freshwater cladocerans, the efficiency of trapping small particles can be predicted from the spacing of setae on their 'filtering' appendages (Brendelberger 1991).

Until the end of the 1970ies, this mechanical concept of zooplankton grazing was also assumed for 'herbivorous' calanoid copepods. Yet, since Alcaez et al. (1980) first employed high speed video cinematography, the concept of copepod grazing has been revolutionised. Calanoid copepods have been shown to remotely detect their prey (Bundy et al. 1998) and selectively ingest particles on the basis of palatability (DeMott 1988), not size alone. Although copepods may ingest particles as small as  $\sim 4\ \mu\text{m}$  due to a passive feeding mode (Vanderploeg and Paffenhöfer 1985), minimum particle size usually lies beyond  $\sim 10\ \mu\text{m}$  (Harris 1982, Berggreen et al. 1988). Thus, copepod grazing differs from both, cladoceran and appendicularian grazing in that copepods may actively maximise prey ingestion at a given foraging effort in accordance with optimal foraging theory (DeMott 1989). Moreover, they may actively avoid 'noxious' algae (Engström et al. 2000).

In the mesocosm studies, it was hypothesised that major zooplankton taxa would select particles primarily on the basis of size. While freshwater *Daphnia* and marine appendicularians were expected to reduce small particles ( $<30\ \mu\text{m}$  and  $<5\ \mu\text{m}$ , respectively), calanoid copepods were predicted to generally impact large ( $>30\ \mu\text{m}$ ) phytoplankton, thus maximising dietary gain at a determined foraging effort. However, some phytoplankton taxa within the edible size range of copepods were likely to be excepted from grazing, as copepods may reject them for reasons of palatability. In this way, major zooplankton taxa within each system (freshwater, brackish and marine) may prove complementary in their impact on phytoplankton and have functional counterparts occupying similar grazing niches in other systems: Calanoid copepods as grazers of larger phytoplankton and mechanical sievers (cladocerans, appendicularians) as grazers of smaller algae.

Primarily the grazing impact of zooplankton on phytoplankton was assessed. Yet, due to potential indirect effects via trophic cascades, I also determined effects on other potential prey items, such as ciliates and rotifers in all, but the first mesocosm experiment. The analysis of zooplankton effects on food web structure was, moreover, a prerequisite in order to interpret nitrogen stable isotope patterns of zooplankton species (Chapter IV), and to understand the effects of different zooplankton guilds on the stoichiometry of their diets (Chapter V).

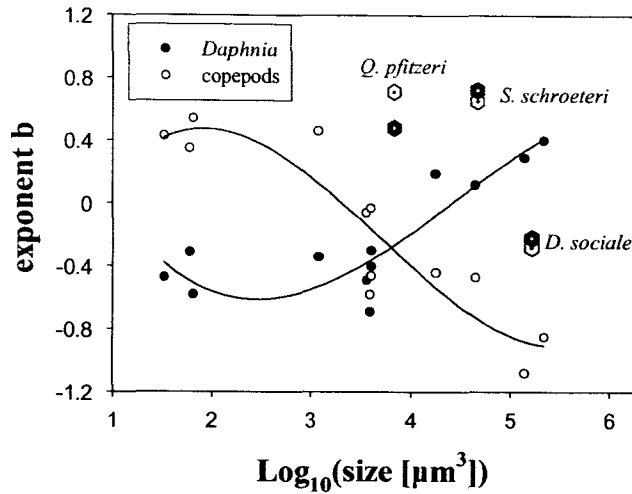
### III.2 Schöhsee: Results and discussion

The Schöhsee experiment was the longest mesocosm study and lasted for approximately three weeks (Table 1). The analysis of the impact of *Daphnia* and copepods on phytoplankton was performed for cell counts from 17 August. At this point, bags of the copepod treatment still showed negligible contamination by *Daphnia* ( $<2 \text{ ind L}^{-1}$ ) individuals.

Typically, the summer phytoplankton community in mesotrophic Schöhsee was diverse. The analysis on 17 August showed that *Daphnia* had a significantly negative impact on most phytoplankton species small in size ( $<4000 \mu\text{m}^3$ , Table 4). In contrast, copepods had a positive impact on small phytoplankton, yet negatively affected medium to large sized phytoplankton species ( $>4000 \mu\text{m}^3$ ). With the exception of gelatinous green algae (*Quadrigula pfitzeri*, *Sphaerocystis Schroeteri*) and the colonial chrysophyte *Dinobryon sociale*, the impact of zooplankton on phytoplankton species was thus largely explained by phytoplankton size (Figure 2).

**Table 4:** Impact of copepods and *Daphnia* on phytoplankton species and bulk parameters on 17 August. Results are from regressions of phytoplankton concentrations as a function of copepod or *Daphnia* seeding densities, respectively, according to the model  $y = ax^b$ . Symbols are: n.s. (not significant,  $p > 0.05$ ), \* ( $0.01 < p < 0.05$ ), \*\* ( $0.001 < p < 0.01$ ), \*\*\* ( $p < 0.001$ ). Table modified after Sommer et al. (2001).

Species/Parameter	higher taxon	size [ $\mu\text{m}^3$ ]	copepods			<i>Daphnia</i>		
			b	$r^2$	p	b	$r^2$	p
Unidentified nanoflagellates $<5 \mu\text{m}$	-	33	0.43	0.78	**	-0.47	0.78	**
<i>Stephanodiscus parvus</i>	diatoms	60	0.35	0.86	***	-0.31	0.75	**
<i>Rhodomonas minuta</i>	cryptophytes	65	0.54	0.76	**	-0.58	0.76	**
<i>Cryptomonas spp.</i>	cryptophytes	1200	0.46	0.87	***	-0.34	0.77	**
<i>Phacotus lenticularis</i>	chlorophytes	3600	-	-	n.s.	-0.49	0.63	*
<i>Rhizochrysis spp.</i>	chrysophytes	3900	-0.58	0.52	*	-0.69	0.78	**
<i>Stephanodiscus alpinus</i>	diatoms	4000	-	-	n.s.	-0.40	0.68	**
<i>Cryptomonas rost.</i>	cryptophytes	4000	-0.46	0.85	***	-0.31	0.69	**
<i>Quadrigula pfitzeri</i>	chlorophytes	6800	0.71	0.58	*	0.48	0.59	*
<i>Peridinium bipes</i>	dinoflagellates	18000	-0.44	0.83	***	0.19	0.77	**
<i>Ceratium hirundinella</i>	dinoflagellates	45000	-0.47	0.80	***	0.12	0.76	**
<i>Sphaerocystis Schroeteri</i>	chlorophytes	47700	0.65	0.87	***	0.72	0.88	***
<i>Microcystis spp.</i>	cyanobacteria	141000	-1.08	0.87	***	0.29	0.71	**
<i>Dinobryon sociale</i>	chrysophytes	165000	-0.28	0.82	***	-0.22	0.52	*
<i>Anabaena flos-aquae</i>	cyanobacteria	220000	-0.85	0.90	***	0.40	0.77	**
chl a [ $\mu\text{g L}^{-1}$ ]	-	-	-	-	n.s.	-	-	n.s.
total biomass [ $\mu\text{g C L}^{-1}$ ]	-	-	-	-	n.s.	-	-	n.s.



**Figure 2:** Impact of *Daphnia* and copepods as indicated by the exponent *b* (given in Table 4) on  $\text{Log}_{10}$ -converted phytoplankton size. Polynomial third-order functions are:  $y=1.37-1.85x+0.52x^2-0.04x^3$  (*Daphnia*:  $r^2 = 0.81$ ,  $p<0.05$ ) and  $y=-1.18+1.95x-0.69x^2+0.06x^3$  (copepods:  $r^2 = 0.84$ ,  $p<0.05$ ). Phytoplankton species indicated with larger, dotted symbols were excluded from regressions. Modified after Sommer et al. (2001).

The fact that gelatinous green algae were positively impacted by both zooplankton was possibly due to active avoidance by copepods, and their low digestibility and uptake of nutrients during gut passage for *Daphnia* (Porter 1976). In turn, large colonial *D. sociale* were negatively impacted, also by *Daphnia*. Though large as a colony ( $165\,000\ \mu\text{m}^3$ ), *D. sociale* may easily disrupt and as single cells ( $175\ \mu\text{m}^3$ ) be well within the size range ingested by *Daphnia*.

On 17 August, both copepods and *Daphnia* showed no significant impact on total phytoplankton biomass or bulk chl *a* content (Table 4). This indicates that negative impacts on certain phytoplankton species was partially compensated for by additional growth of positively affected phytoplankton. The impact of zooplankton on phytoplankton biomass on 28 August, when treatments had become severely contaminated by *Daphnia*, was analysed using a multiple regression analysis with stepwise variable selection (analysis in Sommer et al. 2001). It showed that the combined impact of copepods and *Daphnia* had a significantly negative impact on total phytoplankton biomass. Thus, copepods and *Daphnia* in the Schöhsee mesocosm experiment were complementary in their impact on phytoplankton with respect to both, phytoplankton size and total phytoplankton biomass. This finding was recently corroborated by a study in Lake Biwa, Japan, where the calanoid copepod *Eodiaptomus japonicus* and the cladoceran *Daphnia galeata* were found to similarly impact large or small particles, respectively (Yoshida et al. 2001)



### III.3 Hopavågen: Results and discussion

The Hopavågen food web may be characterised as extremely oligotrophic at the time of experimentation (see also Chapter V.3). It was dominated by heterotrophic ciliates and nanoflagellates, which both contributed more strongly to seston C content (~5 to 10% and ~7 to 11%, respectively) than diatom biomass (~3 to 6%).

Copepods had a strong negative impact on heterotrophic ciliates, diatoms, the coccolithophorid *Emiliana huxleyi* and the dinoflagellate *Gymnodinium* sp. (Table 5). There was no significant impact on the cryptophyte *Teleaulax acuta*. Nanoflagellates, however, were positively affected by copepods. Nanoflagellate abundance was significantly negatively correlated with total ciliate biovolume ( $y=3965+12440e^{-0.001x}$ ,  $r^2 = 0.65$ ,  $p<0.001$ ), which suggests that the increase of nanoflagellates with copepod density was a result of reduced ciliate grazing pressure via a trophic cascade 'copepod-ciliates-nanoflagellates'. The increase of total chl a with mean copepod density paralleled the increase of nanoflagellates, as nanoflagellates contributed strongly to total chl a (linear regression:  $\text{chl a} = 0.9+10^{-3} \times \text{nanoflagellates}$ ,  $r^2 = 0.85$ ,  $p<0.001$ ). The total biomass of all plankton taxa was not significantly impacted by copepods.

**Table 5:** Impact of copepods on plankton taxa and bulk parameters on 19 July. Results are from regressions of cell concentrations as a function of copepod mean densities (16 and 19 July) according to the model  $y = ax^b$ . Symbols are: n.s. (not significant,  $p>0.05$ ), \*\*\* (0.0001< $p$ <0.001), \*\*\*\* ( $p<0.0001$ ).

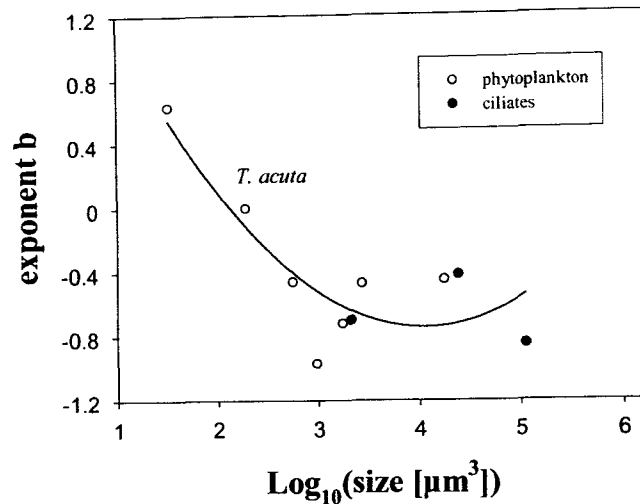
Species/Parameter	size [ $\mu\text{m}^3$ ]	copepods		
		b	$r^2$	p
Unidentified nanoflagellates <5 $\mu\text{m}$	33	0.63	0.68	***
cryptophytes				
<i>Teleaulax acuta</i> (10-15 $\mu\text{m}$ )	190	-	-	n.s.
prymnesiophyte				
<i>Emiliana huxleyi</i>	560	-0.46	0.87	****
diatoms				
<i>Leptocylindrus minimus</i>	980	-0.97	0.93	****
<i>Guinardia delicatula</i>	2750	-0.47	0.72	***
<i>Cerataulina pelagica</i>	17670	-0.45	0.78	***
dinoflagellates				
<i>Gymnodinium</i> sp. (~15 $\mu\text{m}$ )	1770	-0.72	0.96	****
ciliates				
small (~10-25 $\mu\text{m}$ )	2150	-0.70	0.73	***
medium (25-50 $\mu\text{m}$ )	24430	-0.42	0.71	***
large (>50 $\mu\text{m}$ )	113100	-0.85	0.95	****
chl a [ $\mu\text{g L}^{-1}$ ]		0.32	0.77	***
total biomass [ $\mu\text{g C L}^{-1}$ ]		-	-	n.s.

The impact of *O. dioica* was analysed using multiple linear regressions with stepwise selection of the independent variables (forward selection). This was done because a negative impact was expected for nanoflagellates, which both appendicularians (Flood et al. 1992, Sommer et al. 2000) and heterotrophic ciliates (which were highly abundant in all bags) are known to feed on. After logarithmic transformation, appendicularian, total ciliate, and the sum of ciliate and appendicularian biomass [ $\mu\text{g C L}^{-1}$ ] were used as independent variables, and concentrations of plankton taxa [ $\text{cells ml}^{-1}$ ] as dependent variables. Significant results were only found for nanoflagellates and the coccolithophorid *E. huxleyi* (Table 6). As indicated by regression analysis, *O. dioica* had no significant impact on nanoflagellates. Instead, ciliate biomass significantly explained most of the variability of nanoflagellate concentrations ( $r^2 = 0.80$ ), which supports the strong trophic link between ciliates and nanoflagellates found in the copepod treatment. Regression analysis suggested a positive impact of ciliates on *E. huxleyi*. This may possibly be the result of increased growth due to enhanced nutrient regeneration by ciliates.

**Table 6:** Results of significant stepwise linear regressions of plankton taxa on appendicularian, ciliate and the sum of appendicularian and ciliate biomass from 22 July (Forward selection, F-to-Enter = 4.0). Appendicularian and ciliate biomass was calculated assuming 25% of adult appendicularian biomass ( $3.2 \mu\text{g C ind}^{-1}$ , Table 10), and according to Putt and Stoecker (1989), respectively. The independent variables were logarithmically transformed [ $\text{Log}_{10}(\text{appendicularian biomass} + 1)$  and  $\text{Log}_{10}(\text{total ciliate biomass})$ ] in order to apply linear regressions. Symbols are: n.s. (not significant,  $p > 0.05$ ), \* ( $0.01 < p < 0.05$ ), \*\* ( $0.001 < p < 0.01$ ).

Taxon	<i>O. dioica</i>		ciliates		<i>O. dioica</i> + ciliates		$r^2$
	coefficient	<i>p</i>	coefficient	<i>p</i>	coefficient	<i>p</i>	
Unidentified nanoflagellates	-	n.s.	-1.1	**	-	n.s.	0.80
<i>Emiliana huxleyi</i>	-	n.s.	0.76	*	-	n.s.	0.63

As in the Schöhsee copepod treatment, particle size was significantly correlated to the exponent  $b$  (Figure 3). Copepods, thus, had a positive impact on small particles (nanoflagellates  $< 5 \mu\text{m}$ ) and a negative impact on large particles ( $> 500 \mu\text{m}^3$ ). In contrast to the Schöhsee, however, copepods in the Hopavågen experiment shifted to grazing on smaller particles, as a neutral response ( $b = 0$ ) was here found for particles smaller in size ( $\sim 200 \mu\text{m}^3$ ) than in the Schöhsee experiment ( $\sim 1500 \mu\text{m}^3$ ). This was possibly due to the lower (phytoplankton) diversity of the food web.



**Figure 3:** The impact of copepods as indicated by the exponent  $b$  (given in Table 5) on  $\text{Log}_{10}$ -converted sizes of plankton taxa. The polynomial second-order function was fitted to all data for all plankton taxa:  $y = -2.6 - 1.6x + 0.2x^2$  ( $r^2 = 0.72$ ,  $p < 0.05$ ). For the cryptophyte *T. acuta*, the exponent  $b$  value was set to zero (neutral response).

Marine oligotrophic systems are found in permanently stratified (sub)tropical open oceans and during the temperate summer, when nutrient concentrations are not detectable and primary production is fuelled via zooplankton nutrient regeneration (Cushing 1989). Under such conditions, pico and nanoplankton are better competitors for nutrients than diatoms, and support food webs dominated by heterotrophic ciliates. The strong impact of copepods on ciliates and the increase of nanoflagellates - presumably via a trophic cascade - are thus consistent with predictions of C flow in marine oligotrophic systems (Azam et al. 1983). Although appendicularians are known to efficiently filter marine bacteria (King et al. 1980), and may show extremely high clearance rates (Bedo et al. 1993), no impact was found for *O. dioica* on nanoflagellates. One possible explanation may be that - on a short time-scale (hours) - ciliates basically respond numerically and appendicularians in somatic growth to changes in nanoflagellate concentrations. Therefore, analyses based on grazer densities, are more likely to yield significant responses for ciliates than appendicularians.

#### III.4 Kiel Fjord: Results and discussion

As in the Schöhsee mesocosm, phytoplankton diversity was high, and composition typical for the smaller autumn bloom dominated by dinoflagellates in Kiel Bight (Lenz 1981). Similarly, water temperatures were high ( $\sim 20^\circ\text{C}$ ), so that intense and rapid development of zooplankton was expected.

The high number of copepods recruiting from nauplii and copepodites thereby probably suppressed the development of appendicularians, as copepods are likely to feed on *O. dioica* juveniles (Sommer et al. 2003b). Peculiarities to this mesocosm experiment were that zooplankton was dominated by one species, *Acartia clausi* (>90%), and that the increase of copepods (*A. clausi*) in one bag - termed bag 24 - was unusually high. This strong increase from initially 7 ind L<sup>-1</sup> on 4 September to 303 ind L<sup>-1</sup> on 13 September occurred rapidly and undetected by the 3-day sampling scheme. The preceding copepod density on 10 September was only 14 ind L<sup>-1</sup>. As a consequence, this bag was practically devoid of any phytoplankton >5 µm on 13 September, except for low concentrations (2 cells ml<sup>-1</sup>) of the diazotrophic cyanobacterium *Nodularia spumigena* (see below). For this reason and due to the high variability of initial phytoplankton concentrations, the impact of copepods was here determined from the analysis of growth rates, not cell concentrations (Chapter II.4).

On a short-term, copepods had a negative impact on large, solitary diatoms (*Coscinodiscus* sp., *Ditylum brightwellii*) and a positive impact on chain-forming diatoms (*Dactyliosolen fragilissimus*, *Cerataulina pelagica*), smaller in cell size (Table 7). Dinoflagellates were generally not or negatively affected (*Protoperidinium* sp., *Ceratium fusus*). A positive impact was detected for what was tentatively identified as a dinoflagellate cyst. It could, however, not be ascribed to any dinoflagellate species. Both, large ciliates and the rotifer *Synchaeta* sp. were negatively affected, whereas small ciliates were positively impacted by copepods. Abundances of small ciliates were not significantly correlated ( $p > 0.4$ , power regressions) to abundances of large ciliates ( $r^2 = 0.16$ ) or of the rotifer *Synchaeta* sp. ( $r^2 = 0.01$ ) on 7 September (not shown). This indicates that the increase of small ciliates with copepod density was probably not the result of release from predation pressure by large ciliates and/or rotifers via a trophic cascade. Rather, bottom-up 'fuelling' of protists and bacteria via nutrient regeneration by copepods seem a more probable explanation. Due to the high content of detritus, concentrations of nanoflagellates could not be determined in the counting chambers.

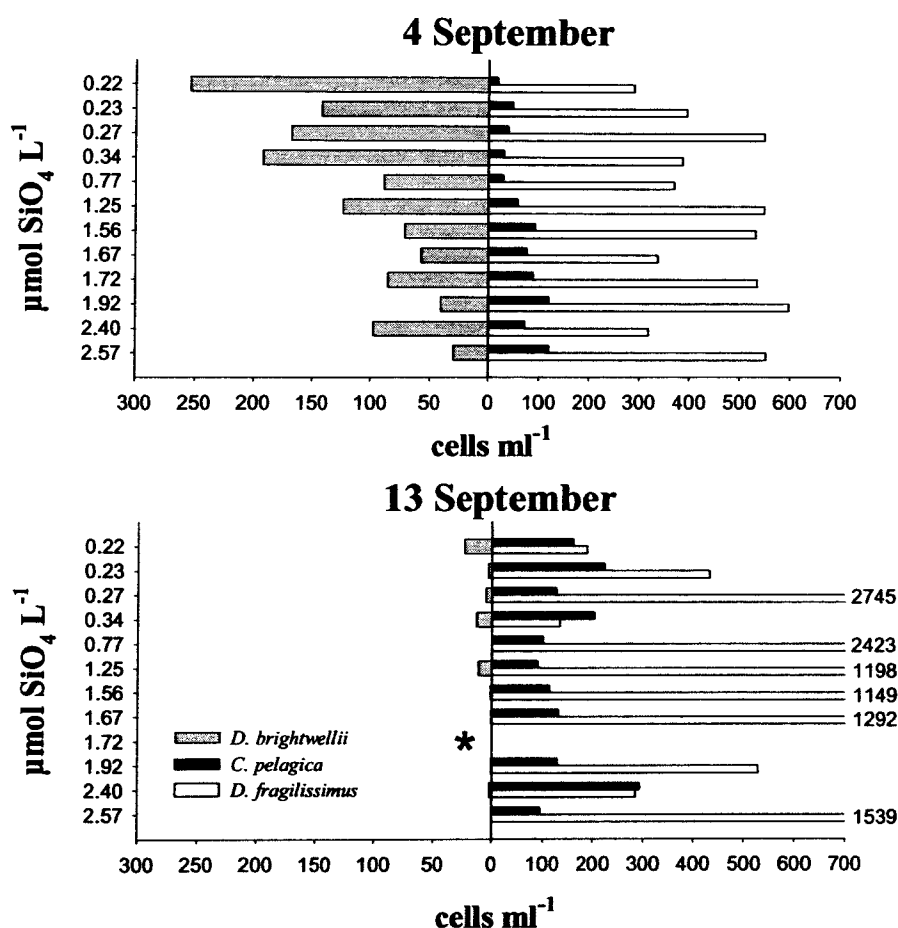
The assessment of long-term impacts was often not possible, as many taxa were already absent by the end of the experiment. A positive impact was observed for *D. fragilissimus*, the 'dinoflagellate cyst', and small ciliates. In turn, large solitary diatoms (*Coscinodiscus* sp., *D. brightwellii*) were negatively affected. Thus, these organisms were consistently positively or negatively affected (compare short and long term impacts). The positive increase of total biomass (including ciliates, yet excluding rotifers) with copepod abundance was mainly attributable to the increase of *D. fragilissimus*. Its contribution to total biomass increased from 27 to 74% on 4 September to 32 to 93% on 13 September, depending on copepod abundance. The strong increase of *D. fragilissimus* was, however, not paralleled by increases of chl a, as these were not correlated with copepod abundance (Table 7). This may be probably due to a low chl a content of *D. fragilissimus*. In terms of cell numbers, *D. fragilissimus* increased from initially <600 cells ml<sup>-1</sup> to >2000 cells ml<sup>-1</sup> in many bags (see also Figure 4).

**Table 7:** The short-term (4 to 7 September) and long-term (4 to 13 September) impact of copepods on the food web. Linear regressions were performed on growth rates as a function of  $\text{Log}_{10}$ -transformed mean copepod abundances. Data from bag 24 were excluded from long-term regression analyses. Abbreviations and symbols are: n. app. (not applicable, due to absence in most bags), n.s. (not significant,  $p>0.1$ ), \* ( $0.05<p<0.1$ ), \*\* ( $0.005<p<0.05$ ), \*\*\* ( $0.0005<p<0.005$ ).

Species/Parameter	max. dimension	4 to 7 September			4 to 13 September		
		slope	$r^2$	$p$	slope	$r^2$	$p$
cryptophytes							
<i>Tetraselmis</i> sp.	9	-	-	n.s.	-	-	n.app.
<i>Rhodomonas</i> sp.	13	-	-	n.s.	-	-	n.app.
diatoms							
<i>Dactyliosolen fragilissimus</i>	80	0.30	0.50	**	0.35	0.58	**
<i>Cerataulina pelagica</i>	88	0.38	0.30	*	-	-	n.s.
<i>Pseudo-nitzschia</i> sp.	97	-	-	n.s.	-0.49	0.76	***
<i>Coscinodiscus</i> sp.	150	-1.94	0.45	**	-1.84	0.68	**
<i>Ditylum brightwellii</i>	175	-0.56	0.52	**	-1.45	0.36	*
dinoflagellates							
<i>Prorocentrum minimum</i>	17	-	-	n.s.	-	-	n.s.
<i>Katodinium</i> sp.	18	-	-	n.s.	-	-	n.s.
(?) cyst	35	0.95	0.43	**	0.42	0.46	**
<i>Protoperdinium</i> sp.	40	-2.91	0.61	**	-	-	n.app.
<i>Dinophysis acuminata</i>	73	-	-	n.s.	-	-	n.app.
<i>Prorocentrum micans</i>	68	-	-	n.s.	-0.16	0.40	**
<i>Ceratium tripos</i>	155	-	-	n.s.	-	-	n.s.
<i>Ceratium fusus</i>	240	-0.19	0.69	***	-	-	n.s.
cyanobacteria							
<i>Nodularia spumigena</i> : cells $\text{mL}^{-1}$	-	-0.68	0.43	**	-	-	n.s.
<i>Nodularia spumigena</i> : colonies $\text{L}^{-1}$	-	-0.54	0.55	**	-	-	n.s.
ciliates							
small, ~10-25 $\mu\text{m}$	20	0.99	0.58	**	0.55	0.67	***
large, ~30-60 $\mu\text{m}$	45	-4.20	0.79	***	-	-	n.app.
rotifer <i>Synchaeta</i> sp.: ind $\text{L}^{-1}$	200	-1.04	0.31	*	-	-	n.app.
chl a [ $\mu\text{g L}^{-1}$ ]		-	-	n.s.	-	-	n.s.
total biomass [ $\mu\text{g C L}^{-1}$ ]		0.18	0.52	**	0.28	0.66	**

Interestingly, the high initial variability in diatom concentrations was linked to concentrations of free silicate (Figure 4). In mesocosm bags, where silicate concentrations were lowest, concentrations of *D. brightwellii* were highest, and conversely, those of *C. pelagica* lowest. Linear correlations of cell concentrations with silicate were significant for both, *D. brightwellii* ( $r^2 = 0.67$ ,  $p<0.05$ .  $y=191-63x$ ) and *C. pelagica* ( $r^2 = 0.75$ ,  $p<0.05$ .  $y=24+34x$ ), yet not for *D. fragilissimus*. Also, there was a significantly negative relationship between cell concentrations of *D. brightwellii* and *C. pelagica* ( $r^2 = 0.80$ ,  $p<0.05$ .  $y=141-1.0x+0.002x^2$ ). This indicates that *D. brightwellii* was probably a strong competitor for silicate.

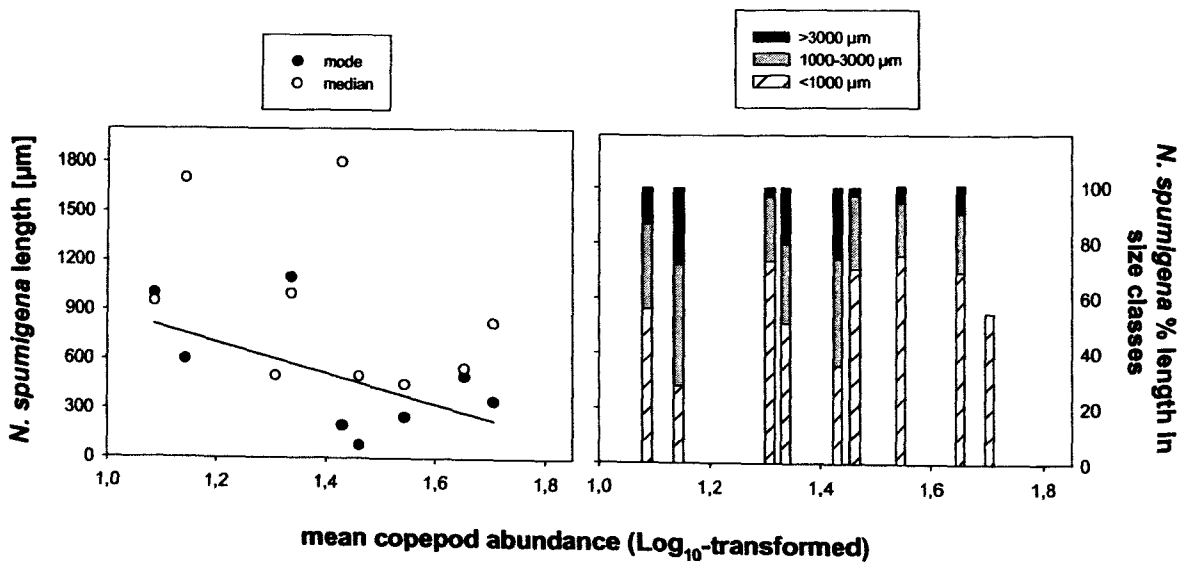
By 13 September, *D. brightwellii* had decreased in concentration in all bags, with copepods apparently contributing to this decrease (Table 7). Conversely, *C. pelagica* and, especially, *D. fragilissimus* had increased in concentration in all bags, yet also here in dependence of copepod abundance (Table 7). Thus, it seems that the strong development of *D. fragilissimus* was favoured by at least two factors: First, the overall decrease in cell concentrations over time and remineralisation of silicate bound in *D. brightwellii*, a process which was enhanced by copepod grazing. Second, enhanced growth with copepod abundance, possibly due to nutrient regeneration by copepods (see Chapter V.3) in combination with low grazing pressure or grazing avoidance by *A. clausi*, and the release from additional grazing pressure via a trophic cascade 'copepods-rotifers/ciliates-diatoms'. A combination of these factors is, of course, also possible.



**Figure 4:** Start (4 September) and final (13 September) concentrations [cells ml<sup>-1</sup>] of the most abundant diatom species *Ditylum brightwellii*, *Cerataulina pelagica* and *Dactyliosolen fragilissimus*. Data (bars) are arranged according to initial concentrations of silicate. Numbers to the right of bars for *D. fragilissimus* on 13 September are final cell concentrations. \* denotes bag 24.

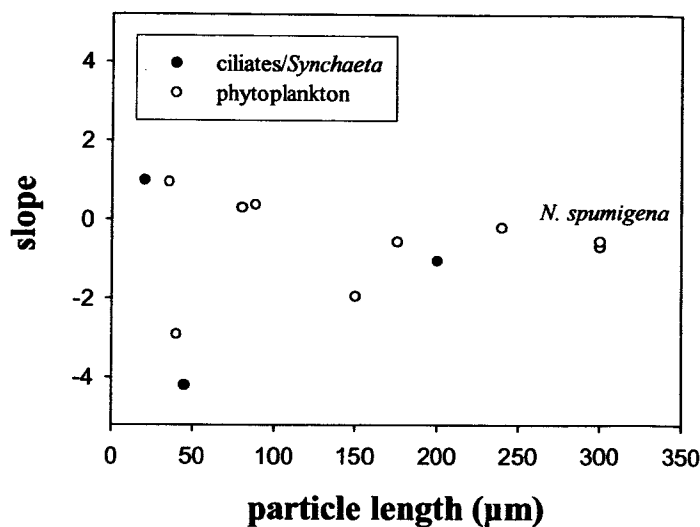
The impact of *A. clausi* on the diazotrophic cyanobacterium *N. spumigena* was ambiguous. At initially low concentrations (<40 cells ml<sup>-1</sup>) copepods had a negative impact on the number of trichomes and, consequently, of cell concentrations (Table 7). Yet, by the end of the experiment, when *N. spumigena* had increased to >1200 cells ml<sup>-1</sup> and >3000 colonies L<sup>-1</sup> in many bags, an impact by *A. clausi* was no longer apparent.

In order to account for the possible reduction of trichome length due to ‘tasting’ by zooplankton (‘filament clipping’: Turner et al. 1998), I assessed the frequency distribution of *N. spumigena* trichome lengths on 13 September (Figure 5). Both, the modes and medians of *N. spumigena* trichome lengths were not significantly correlated with mean copepod densities, although the modes of trichome length were smaller at high copepod densities (>30 ind L<sup>-1</sup>). Equally, the relative distribution of trichomes in three different size classes revealed no obvious effect of copepods on the length of *N. spumigena* trichomes. This indicates that copepod grazing on *N. spumigena* was probably negligible, at least in the long run. The strong reduction of *N. spumigena* in bag 24 (from 144 cells ml<sup>-1</sup> on 7 September to 2 cells ml<sup>-1</sup> on 13 September), however, shows that *A. clausi* may feed and successfully reproduce on *N. spumigena*.



**Figure 5:** Trichome length of *N. spumigena* [μm] as a function of Log<sub>10</sub>-transformed mean copepod abundances on 13 September showing the modes and medians (left), and their relative distribution in three size classes (right). Samples of bags in which <20 trichomes were counted in a 50 ml settling chamber were excluded (n=3). The linear regression fitted to the modes of *N. spumigena* trichome length is:  $y = 1832 - 940 x$ ,  $r^2 = 0.33$ ,  $p=0.11$ ).

Plotting slope values of the plankton taxa that were significantly affected by copepods (Table 7), showed that the impact by copepods was moderately negative ( $\sim 0$  to  $-2$ ) for food particles  $>100\ \mu\text{m}$  (in maximal dimension), but both positive or highly negative for particles  $<100\ \mu\text{m}$  (Figure 6). The large number of phytoplankton that were not affected, and also the sometimes varying impact (e.g. *N. spumigena*, *Pseudonitzschia* sp.) indicate that feeding by *A. clausi* was not primarily based on size.



**Figure 6:** Short-term impact of copepods (as indicated by the slope of linear regressions in Table 7) on particle length (maximal dimension) of plankton taxa. The length of *N. spumigena* trichomes varied from 12 to 690  $\mu\text{m}$  (4 and 7 September) and - for illustration - is here plotted as 300  $\mu\text{m}$  (both, slopes values of cell concentration and trichome length).

The fact that copepods in the Kiel Fjord mesocosm were almost exclusively represented by *A. clausi* allows for food web effects to be discussed under the biology of this species. It is well known that *A. clausi* may at times feed strongly as a carnivore. Strong predation on ciliates, rotifers and heterotrophic dinoflagellates, often exceeding grazing rates on phytoplankton, are well documented in the literature (Stoecker and Sanders 1985, Stoecker and Capuzzo 1990, Fessenden and Cowles 1994, Stoecker and Egloff 1987, Kleppel et al. 1998). Also, observations of *Acartia* swimming behaviour suggest a relatively carnivorous diet: motionless sinking with short jumps, ideal for 'ambushing' motile prey (Tiselius and Jonsson 1990). When ciliates are scarce (or immotile prey abundant) *Acartia* may though switch to a more herbivorous, stationary foraging mode (Kjørboe et al. 1996).

Therefore, the negative impact on heterotrophic microplankton, and large motile dinoflagellates in this mesocosm are consistent with reports of *Acartia* feeding. Selectivity for certain phytoplankton species of dinoflagellates and, especially diatoms, was pronounced. Colonial diatoms were consistently positively, whereas large solitary diatoms consistently negatively impacted. Possibly, the colonial *D.*



*fragilissimus* was unpalatable and even harmful, as in recent years, marine diatoms have been debated as a – at times - potentially poor or even harmful diet for copepods (Ban et al. 1997, Jónasdóttir et al. 1998, Ianora et al. 1999). Also, the unclear impact of *Acartia* on the filamentous cyanobacterium *Nodularia* is an unresolved question in marine planktology, as toxic strains of *N. spumigena* may be avoided (Engström et al. 2000) or readily ingested despite their toxicity (Schmidt and Jónasdóttir 1997, Koski et al. 2002). In this way, the Kiel Fjord mesocosm showed aspects of many topics currently debated in marine literature. It, however, clearly demonstrated that other factors, more important than particle size, may provide the basis for copepods to ‘decide’ whether a certain algae is ingested or not.

### III. 5 Summary and conclusions

The goals concerning the analysis of zooplankton grazing on phytoplankton were two-fold. First, to assess the grazing impact of at least two major zooplankton taxa differing in feeding ecology within a system. Second, to compare these impacts across systems, where similar functional groups of zooplankton were expected to occupy similar grazing niches.

Obviously, these ambitious goals could not be fully met here. Only in the freshwater mesocosm, density gradients of two different zooplankton guilds could be established *a priori*. This was possible because copepods were taken from natural populations in the lake and *Daphnia* from stock cultures in the laboratory. Similarly, copepods of the marine mesocosms originated from natural assemblages. Yet, appendicularian treatments were subject of ‘chance’, as they were established by reducing copepods in expectation of appendicularians to develop. Although the appendicularian *O. dioica* did in fact develop in one of the marine mesocosms, the analysis of its impact was complicated by the high number of heterotrophic ciliates present together with *O. dioica* in the mesocosm bags. This is because appendicularians are - in a way - an exceptional group of (meso)zooplankton. They feed within the same particle size range as ciliates (Gorsky and Fenaux 1998), but - unlike ciliates - are themselves preyed upon by fish larvae (Shelbourne 1962; Ryland 1964), and therefore represent a direct trophic link from bacteria to fish.

Thus, the comparison of the grazing impact of major zooplankton within a system is restricted to the Schöhsee experiment. Here, the results were very clear and as predicted. While copepods had a negative impact on large phytoplankton, *Daphnia* negatively affected smaller species. Both proved to be complementary in their impact on summer phytoplankton assemblages, and only the combined grazing of copepods and *Daphnia* significantly reduced phytoplankton biomass. Recent evidence (Yoshida 2001) support this finding. The three algae found to be exceptions to this pattern (gelatinous green algae and *D. sociale*) may either profit from enhanced nutrient cycling by zooplankton paired with low digestibility (*Daphnia*) and active avoidance (copepods), or may be ingested after disruption of their colonies.

A comparison across systems may be made for copepods, since copepod treatments were successfully installed in all mesocosm studies. The impact of copepods was analysed under the aspect of particle size, given that copepods may act as optimal foragers (DeMott 1989). Copepods met the predictions of feeding on larger particles in the Schöhsee and Hopavågen experiments, as these were negatively affected. In turn, small particles - especially nanoflagellates, which are beyond the edible size-limit for copepods ( $\sim 4 \mu\text{m}$ , Vanderploeg and Paffenhöfer 1985) - were favoured by copepods. In the Hopavågen experiment, copepods 'shifted' their negative impact to even smaller particles than in the Schöhsee experiment. Clearly, phytoplankton taxon or potential toxicity (e.g. cyanobacteria) were not determinants of phytoplankton susceptibility to grazing by copepods. For example, diatoms were impacted, both positively (Schöhsee, Kiel Fjord) and negatively (Hopavågen, Kiel Fjord), even if it was the same species (*C. pelagica*). Note, however, that they differed strongly in size (Tables 4 and 5). Also, long-term impacts on potentially toxic cyanobacteria were ambiguous (*N. spumigena*: Kiel Fjord) or clearly negative (*Anabaena*, *Microcystis*: Schöhsee). Only gelatinous green algae in freshwater were clearly positively affected, but their absence in the marine mesocosms makes a generalisation difficult. Thus, while copepods may favour small particles, the selective impact on larger particles, especially evident in the Kiel Fjord mesocosm, was clearly not in all cases based on particle size alone.

Such selectivity may be based on aspects of food quality (e.g. the cellular N content: Butler et al. 1989). DeMott (1988) clearly demonstrated that calanoid copepods decide on the 'taste' of particles whether they ingest them or not. Additionally, interspecific differences in the foraging modes of calanoid copepods may determine their capability of supplementing their diets with heterotrophs (Greene 1988, see Chapter IV.4). Foraging modes of calanoid copepods may also change with the relative abundance of phytoplankton (Kiørboe and Saiz 1995, Kiørboe et al. 1996, Caparroy et al. 1998). Thus, food web structure - the abundance or scarcity of heterotrophic prey - may also determine the degree of copepod herbivory and, consequently, their impact on phytoplankton. In fact, strong negative impacts on heterotrophic microplankton were observed in the marine mesocosms. And, nitrogen stable isotopes of *E. gracilis* provide indirect evidence that this freshwater calanoid copepod was also strongly carnivorous (see Chapter IV.6).

In conclusion, the impact of herbivorous zooplankton on phytoplankton size was consistent with the proposed hypothesis in the Schöhsee and - for copepods - in the Hopavågen experiments. Although calanoid copepods were found to favour or not to affect small phytoplankton, the impact on large phytoplankton was diverse. It is proposed that, besides their size, the 'taste' of particles and differences in omnivory by calanoid species - dependent on food web structure and, possibly, foraging strategy - are strong determinants of phytoplankton fate.

## IV. Nitrogen stable isotope signatures

### IV.1 Introduction

An animal's nitrogen stable isotope signature ( $\delta^{15}\text{N}$ ) becomes enriched per trophic transfer by ~3 to 4‰ (Minagawa and Wada 1984). Physiologically, it is assumed that peptide bonds containing isotopically 'light'  $^{14}\text{N}$  are preferentially broken during trans and deamination processes (Macko et al. 1986), e.g. during the synthesis of dietary proteins (Bada et al. 1989). Therefore, isotopically 'heavy'  $^{15}\text{N}$  is preferentially retained in the consumer causing an enrichment in its  $\delta^{15}\text{N}$ , while 'light'  $^{14}\text{N}$  is excreted (DeNiro and Epstein 1981). Notably, starvation may equally result in increases of an animal's  $\delta^{15}\text{N}$  (Hobson et al. 1993), as animals are then physiologically 'living on their own flesh'.

Feeding, however, also implies that some fraction of body nitrogen will be replaced by 'new' nitrogen originating from the food source. If the  $\delta^{15}\text{N}$  of the food source changes, the extent of trophic enrichment may be greatly outweighed by the 'adjustment' of the animal's  $\delta^{15}\text{N}$  to dietary  $\delta^{15}\text{N}$ , as 'new' dietary nitrogen is incorporated. This is especially evident, if food sources are experimentally labelled with  $^{15}\text{N}$  causing dramatic increases of the  $\delta^{15}\text{N}$  with trophic transfer (Carman and Fry 2002). Less dramatic changes are seen when diazotrophically fixed nitrogen enters the planktonic food web. Atmospheric nitrogen, which by definition has a  $\delta^{15}\text{N}$  of zero, thus typically causes decreases in the  $\delta^{15}\text{N}$  of plankton size fractions, as it is transferred up the food chain (Rolff 2000).

Given the great flexibility in the diets of omnivorous zooplankton, interspecific differences within this guild may be expected to be high (Vander Zanden and Rasmussen 2001). The original rationale of the analysis of nitrogen stable isotopes in the mesocosm studies was to reveal the relative degree of omnivory by the zooplankton tested. This was based on the assumption that increased carnivory by a zooplankton should result in a relatively higher  $\delta^{15}\text{N}$ . The presence of cyanobacteria in two mesocosm experiments (Schöhsee and Kiel Fjord), however, hampered the interpretation of zooplankton  $\delta^{15}\text{N}$  under this aspect, a circumstance which was realised after the marine mesocosm studies. As some important variables were not measured in the first, pilot mesocosm study (Schöhsee), results from this experiment are presented and discussed last. Although tentative conclusions on the relative degree of herbivory/carnivory of some zooplankton species could only be drawn in one experiment, interesting insights in the transfer of diazotrophic nitrogen were gained in the others.

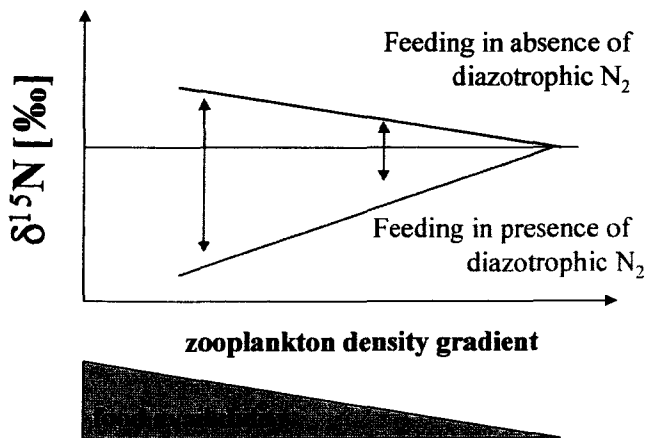
### IV.2 The density-dependence of zooplankton $\delta^{15}\text{N}$

Changes in an animal's  $\delta^{15}\text{N}$  signature basically depend on three factors: the  $\delta^{15}\text{N}$  signature of its diet (1), the velocity of nitrogen exchange (2), and the relative quantity of nitrogen exchanged with respect to total body nitrogen (3). The velocity of nitrogen turnover - as all physiological processes - may be expected to depend primarily on temperature. However, interspecific differences in somatic and population growth may equally result in differing tissue turnover-rates. For example, a rapidly growing, parthenogenetically reproducing zooplankton may 'adjust' more quickly to the isotopic

signature of its diet than a slower growing species (Grey 2000). Finally, the relative amount of nitrogen exchanged within a time unit will determine, how ‘stable’ a consumer’s  $\delta^{15}\text{N}$  signature will be over time. While changes in phytoplankton and zooplankton  $\delta^{15}\text{N}$  may be rapid (within hours to a few days, O’Reilly and Hecky 2002), larger animals, such as fish, may appear unaffected by short-term changes of the  $\delta^{15}\text{N}$  of their food source. This is because only relatively little nitrogen is exchanged with respect to total body tissue nitrogen within a given time.

One factor crucial for the relative quantity of nitrogen exchanged in an animal is the amount of available food. Clearly, food shortage will allow for only little nitrogen exchange (although increases of the  $\delta^{15}\text{N}$  may occur due to nutritional stress: Hobson et al. 1993, Adams and Sterner 2000). Yet, the potential of change in an animal’s  $\delta^{15}\text{N}$  signature may be expected to increase, as more nitrogen is replaced in the animal, or - in other words - the more food is available. Thus, the more of its diet a zooplankton will ingest, the stronger it will ‘approximate’ its  $\delta^{15}\text{N}$  to the  $\delta^{15}\text{N}$  of its diet (+ ~3‰ trophic enrichment), be it isotopically ‘heavy’ (deep-water nitrate: O’Reilly and Hecky 2002) or isotopically ‘light’ (diazotrophic cyanobacteria: Rolff 2000). In the absence of diazotrophic nitrogen, this ‘approximation’ will mean trophic enrichment according to its diet.

In the mesocosm studies, density gradients of zooplankton were established by adding an increasing number of zooplankton to an approximately fixed water volume. This setup allowed for zooplankton to become potentially limited by food - and thus dietary nitrogen – as the availability of food particles gradually decreased with increasing zooplankton densities (Figure 7).



**Figure 7:** Schematic model of nitrogen stable isotope dynamics as a function of zooplankton density, if food is considered limiting to zooplankton grazing.

Principally, zooplankton were expected to show a density-dependent increase in their  $\delta^{15}\text{N}$  as zooplankton density decreased. This was based on the assumption that a low food supply (in the highest density treatments) would result in only marginal changes of zooplankton  $\delta^{15}\text{N}$  with respect to start  $\delta^{15}\text{N}$ , as virtually no or only negligible trophic fractionation may occur. Yet, as the availability of dietary nitrogen increased (at lower zooplankton densities), enrichment of zooplankton  $\delta^{15}\text{N}$  would become gradually stronger due to trophic fractionation. Mathematically, stronger carnivory by a

species would be indicated by a steeper increase of zooplankton  $\delta^{15}\text{N}$ , or a steeper slope of a linear regression fitted to the data. In the highest zooplankton treatments, increases of final zooplankton  $\delta^{15}\text{N}$  over start  $\delta^{15}\text{N}$  may differ due to species-specific tolerances in starvation (Mauchline 1998).

In the presence of diazotrophic cyanobacteria, trophic enrichment may be obscured by the transfer of 'new', isotopically 'light' nitrogen to zooplankton. Thus, zooplankton  $\delta^{15}\text{N}$  were expected to decrease with decreasing zooplankton abundance, yet only if 'new' nitrogen was within dietary particles that were limiting zooplankton grazing. If, for example, diazotrophic cyanobacteria were very abundant and therefore a non-limiting food resource for zooplankton over the entire density gradient, the  $\delta^{15}\text{N}$  of zooplankton may decrease equally in all bags, irrespective of zooplankton abundance.

#### IV.3 Effects of ethanol-fixation on zooplankton $\delta^{15}\text{N}$

The separation of zooplankton on a species level is time-consuming and cannot be performed effectively during the course of an experiment. Therefore fixation is required. Ethanol was chosen for sample fixation, because it has been shown to have no significant effect on the  $\delta^{15}\text{N}$  of *Drosophila* samples, in contrast to formalin preservation (Ponsard and Amlou 1999). In order to account for possible changes of the  $\delta^{15}\text{N}$  of zooplankton, I assessed the effects of ethanol fixation on zooplankton  $\delta^{15}\text{N}$ . Carbon isotope signature ( $\delta^{13}\text{C}$ ) of marine zooplankton were simultaneously measured and are also shown.

The effect of ethanol fixation on zooplankton  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  was tested in May 2002 at the Hopavågen lagoon, Norway. Zooplankton samples were taken in the lagoon with a plankton net of 50  $\mu\text{m}$  mesh size. Half of the samples were sorted immediately under a dissecting microscope ('fresh'), while the other half was fixed with 90% ethanol and sorted 3 days later ('ethanol').

Fixation of zooplankton with 90% ethanol significantly enriched the  $\delta^{13}\text{C}$  of all zooplankton by 0.9 to 1.3‰ (Table 8). This was expected, since ethanol extracts lipids which contain relatively more  $^{12}\text{C}$  and are hence isotopically 'lighter' than other biomass compounds (McConnaughey and McRoy 1979). Especially, copepods may contain large lipid droplets filled with wax esters of which they were visibly devoid after fixation with ethanol. Similarly, however, the  $\delta^{15}\text{N}$  of all zooplankton were enriched after fixation. This was surprising because nitrogen in copepods is mainly present in amino acids of the chitin cuticle and in proteins, the majority of which were thought to be insoluble in ethanol. The enrichment of the  $\delta^{15}\text{N}$  was high for *C. finmarchicus* (1.9‰) and *C. hamatus* (1.4‰), while relatively low for *T. longicornis* (0.4‰). Changes of the  $\delta^{15}\text{N}$  were insignificant for *Podon*. Thus, increases of the  $\delta^{15}\text{N}$  after fixation were species-specific, so that a particular zooplankton species appeared isotopically 'heavier' than another species after fixation, while the opposite was true for 'fresh' samples (compare *C. hamatus* and *T. longicornis*).

**Table 8:** Effects on the  $\delta^{15}\text{N}$  [‰] and  $\delta^{13}\text{C}$  [‰] of different zooplankton taxa after fixation with 90% ethanol. Isotope data are means  $\pm$  1 SD in brackets below. Statistics are results of Student's t-tests. Abbreviations and symbols are: n (number of replicates), Ind. (number of individuals per sample), - (no data), n.d. (not determined), \* ( $p < 0.05$ ), \*\* ( $p < 0.005$ ), n. sign. (not significant). For *Podon*, Ind. was only determined in the ethanol samples (50 ind). *C. finmarchicus* were copepodite stage 5.

<sup>1</sup> indicates that one  $\delta^{13}\text{C}$  sample was lost for *T. longicornis*.

Taxon	n	Ind.	fresh		ethanol		<i>p</i>	
			$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
<i>Centropages hamatus</i>	6	30	6.4 (0.2)	-23.0 (0.1)	7.8 (0.1)	-22.1 (0.1)	**	**
<i>Temora longicornis</i>	6	30	6.8 (0.1)	-23.0 (0.1)	7.2 (0.03)	-22.1 <sup>1</sup> (0.2)	**	**
<i>Calanus finmarchicus</i>	6	10	7.0 (0.1)	- -	8.9 (0.2)	- -	**	-
<i>Podon</i> sp.	3	n. d. (50)	7.0 (0.1)	-23.2 (0.7)	7.3 (0.2)	-21.9 (0.2)	n. sign.	*

This finding has implications for the interpretation of zooplankton  $\delta^{15}\text{N}$  in this study. Interspecific differences in the absolute values need to be interpreted with caution, as species-specific losses of 'light'  $^{14}\text{N}$  may substantially contribute to differences of trophic enrichment between species. In contrast, trophic enrichment inferred from regression analysis over the zooplankton density gradient should be independent from ethanol effects. First, because all zooplankton  $\delta^{15}\text{N}$  samples - start and final samples - were fixed with ethanol. And second, because trophic enrichment was inferred from treatment dependent differences between enclosures and not from absolute zooplankton  $\delta^{15}\text{N}$ .

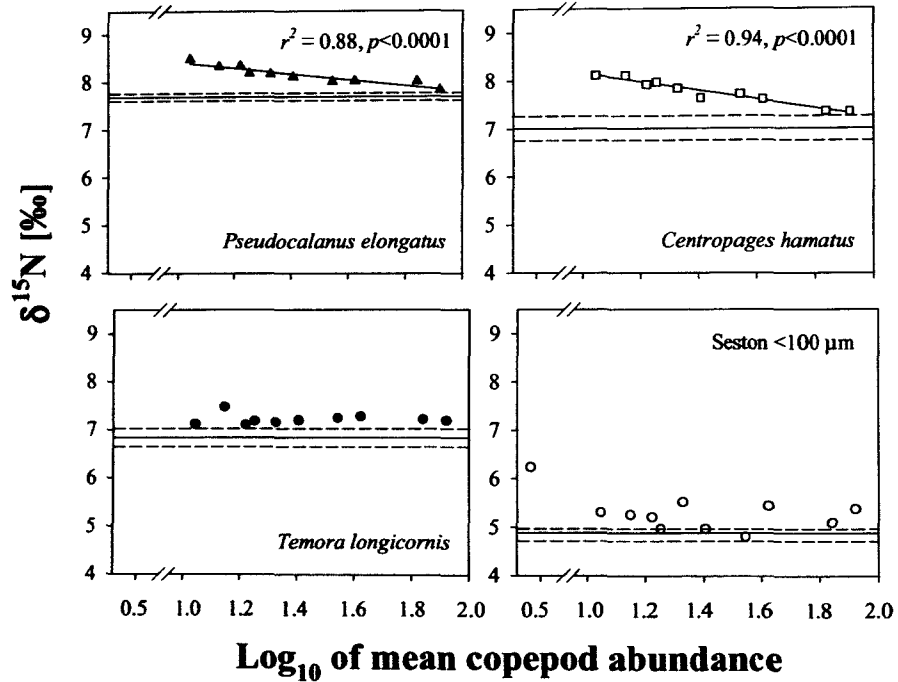
Due to the lack of an alternative fixative, and because separation of live zooplankton was not possible, zooplankton samples were fixed with ethanol in all three mesocosm studies. Recently, Kaehler and Pakhomov (2001) showed that ethanol may similarly increase the  $\delta^{15}\text{N}$  of large marine organisms, but only moderately in contrast to formalin preservation. They concluded - like I may - that ethanol is nevertheless the best choice for the storage and preservation of  $\delta^{15}\text{N}$  samples.

#### IV.4 Hopavågen $\delta^{15}\text{N}$

##### IV.4.1 Results

Final  $\delta^{15}\text{N}$  signatures of the three dominant copepod species in the copepod treatment are shown in Figure 8. Two species of copepods, *Pseudocalanus elongatus* and *Centropages hamatus*, displayed the expected pattern of  $\delta^{15}\text{N}$  signatures along the copepod density gradient. In bags of the highest copepod densities, final  $\delta^{15}\text{N}$  of *P. elongatus* and *C. hamatus* were least enriched (0.1 and 0.4‰, respectively)

with respect to the start  $\delta^{15}\text{N}$ . Yet, as copepod densities decreased, final  $\delta^{15}\text{N}$  of these zooplankton became gradually enriched. The slope value of the linear regression was higher for *C. hamatus* (-0.91) suggesting stronger trophic enrichment, than for *P. elongatus* (-0.62). In contrast, final  $\delta^{15}\text{N}$  of *Temora longicornis* were not correlated with mean copepod abundance, but instead showed an overall enrichment with respect to the start  $\delta^{15}\text{N}$  (~0.4‰). The fact that no correlation was evident for *T. longicornis*  $\delta^{15}\text{N}$  mathematically implied that trophic enrichment was zero, since no slope could be calculated.



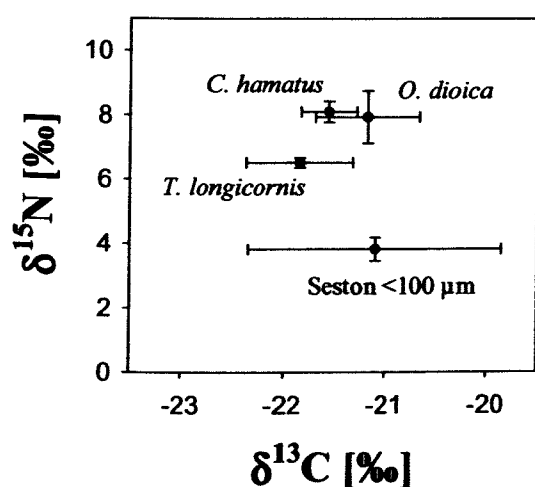
**Figure 8:** Final  $\delta^{15}\text{N}$  signatures [‰] of the copepods *P. elongatus*, *C. hamatus*, *T. longicornis*, and of seston <100  $\mu\text{m}$  in the copepod treatment.  $\delta^{15}\text{N}$  signatures are plotted as a function of  $\text{Log}_{10}$ -transformed mean copepod densities, which were calculated as the arithmetic mean of copepod densities over the experimental period. The mean and  $\pm 1$  SD of start  $\delta^{15}\text{N}$  ( $n = 4$ ) are indicated as solid and dotted horizontal lines, respectively. Replicate measurements of start values are pseudoreplicates of within-variability as they were taken from start populations in the barrel. Linear regressions are:  $y = 9.05 - 0.62x$  (*P. elongatus*) and  $y = 9.07 - 0.91x$  (*C. hamatus*). Note breaks in abscissa.

This may stem from two possibilities. If *T. longicornis* grazing was constrained by food supply in all bags, the overall enrichment of ~0.4‰ would be the result of isotopic enrichment due to starvation. Similarly, the  $\delta^{15}\text{N}$  enrichment of *C. hamatus* and *P. elongatus* in the highest density treatments would be the result of a low food supply (which, however, increased with decreasing zooplankton densities).

Alternatively, overall enrichment in the  $\delta^{15}\text{N}$  of *T. longicornis* may stem from an unlimited food supply, so that food ingestion was equally high in all bags, and therefore, ingestion rates independent from copepod density. The general scarcity of food in the Hopavågen food web, however, indicated that the food supply for copepods was low, even at low copepod densities (see Chapter V.3). Therefore, starvation or an extremely limited food supply for *T. longicornis* seem more probable.

Seston  $\delta^{15}\text{N}$  was not correlated with copepod treatment density (Figure 8). In all treatments with copepods, seston  $\delta^{15}\text{N}$  values were lower (range: 4.8 to 5.5‰) than in the control treatment (6.2‰; data lost for the control replicate). This indicates that 'light'  $^{14}\text{N}$  from copepod tissue was transferred to seston (as isotopically 'light' ammonium, Checkley and Entzeroth 1985) balancing the increases in zooplankton  $\delta^{15}\text{N}$ . This transfer of 'light' copepod  $^{14}\text{N}$  to seston should, however, result in seston  $\delta^{15}\text{N}$  to become isotopically 'lighter' with respect to start values. Yet instead, seston  $\delta^{15}\text{N}$  became enriched in nearly all bags over time. As copepods represented a large portion of total particulate nitrogen (11 to 51% depending on treatment density, see Chapter V.3.2) and generally suffered from high mortality (see Chapter V.3.2), the increases of seston  $\delta^{15}\text{N}$  in bags containing copepods may result from ('heavy') nitrogen from dead individuals collected on the filters. However, relatively higher seston  $\delta^{15}\text{N}$  were not correlated with increased copepod mortality ( $r^2 = 0.01$ ), which would explain the variability in the range of seston  $\delta^{15}\text{N}$  in the copepod bags (4.8 to 5.5‰). Moreover, the highest increase of seston  $\delta^{15}\text{N}$  occurred in the control bag, where copepods were regularly removed. This indicates that isotopically 'light'  $^{14}\text{N}$  was generally lost in all mesocosm bags, possibly to periphyton developing on the enclosure foil or to the DON pool. There are yet no data to support this notion.

Final zooplankton  $\delta^{15}\text{N}$  in the appendicularian treatment were sampled on 28 July. Due to varying densities of appendicularians (and also of copepods), a sufficient number of individuals for zooplankton  $\delta^{15}\text{N}$  samples could not be obtained for all bags. Copepods in this treatment were probably individuals that had escaped the initial removal of zooplankton with net hauls or had recruited from nauplii and copepodites.



**Figure 9:** Final  $\delta^{15}\text{N}$  [‰] of *C. hamatus*, *T. longicornis*, *O. dioica* and of seston in bags of the appendicularian treatment.  $\delta^{15}\text{N}$  are plotted against  $\delta^{13}\text{C}$  signatures. Symbols are means of 4 (*C. hamatus*, *O. dioica* and seston) or 3 (*T. longicornis*) replicate measurements. Bars represent  $\pm 1$  SD. The  $\delta^{15}\text{N}$  of *T. longicornis* is significantly different from both, the  $\delta^{15}\text{N}$  of *O. dioica* and of *C. hamatus* ( $p < 0.05$ , respectively, Tukey's HSD test for unequal sample sizes).



Figure 9 shows that the  $\delta^{15}\text{N}$  of *T. longicornis* was on average 1.6‰ lower than the  $\delta^{15}\text{N}$  of *C. hamatus*. The  $\delta^{15}\text{N}$  of *O. dioica* was practically identical and not significantly different from the  $\delta^{15}\text{N}$  of *C. hamatus* ( $p < 0.05$ , Tukey's HSD test for unequal sample sizes). Both, *C. hamatus* and *O. dioica* were though significantly enriched over *T. longicornis* ( $p < 0.05$ , Tukey's HSD test for unequal sample sizes). Considering that fixation with ethanol may increase the  $\delta^{15}\text{N}$  of *C. hamatus* more strongly (+1.4‰) than the  $\delta^{15}\text{N}$  of *T. longicornis* (+0.4‰: Table 8), inferences from differences in these species'  $\delta^{15}\text{N}$  to differences in their degree of herbivory are difficult to make. Loss of  $^{15}\text{N}$  due to fixation may have implications for the  $\delta^{15}\text{N}$  of *O. dioica*. If isotopic loss is similar for both copepods and appendicularians, then *O. dioica* would be isotopically within the range of omnivorous calanoid copepods. If fixation losses are assumed only for copepods and not for *O. dioica*, then *O. dioica* was isotopically more strongly enriched than copepods (+1.2 to 1.8‰). Alternatively, fixation may have increased the  $\delta^{15}\text{N}$  of *O. dioica* more strongly than copepod  $\delta^{15}\text{N}$ , in which case, *O. dioica* would be isotopically the least enriched species. Whatever the 'true' relationship of unfixed zooplankton  $\delta^{15}\text{N}$ , interspecific differences between appendicularians and omnivorous calanoid copepods may be assumed a lot smaller than for freshwater zooplankton (see Chapter IV. 6).

#### IV.4.2 Discussion: Copepod foraging strategy explains $\delta^{15}\text{N}$

In the Hopavågen food web, heterotrophic ciliates and diatoms basically constituted the only large particles edible by copepods. Both were strongly reduced by the copepod assemblage (Chapter III.3). Given the simplicity of the food web, omnivory by different copepod species may be simplified to the ingestion of varying proportions of diatoms and ciliates (and possibly zooplankton eggs/juveniles). Thus, pure herbivory implied that only diatoms were ingested. If ciliates were to be a supplementary item of a copepod's diet, copepods would require the ability to capture this mobile prey which shows effective escape responses to water motion.

Greene (1988) associated the different patterns of swimming and feeding of calanoid copepods to different diets. On the far ends of a range of different foraging behaviour, herbivorous stationary suspension feeders contrast with strongly carnivorous cruising copepods. In a comparative study, Tiselius and Jonsson (1990) classified several calanoid copepod species on the basis of their foraging behaviour. They found that *T. longicornis* and *P. elongatus* produced strong and wide flow fields advantageous for the feeding on suspended, less motile prey. These flow fields were hydrodynamically 'loud' and thus well perceivable by prey. In contrast, *Acartia*, as a typical ambush predator, generated very low deformation rates, which allows for the hydrodynamically 'quietest' approach of motile prey. Similar to *Acartia*, *Centropages* employs a more predatory 'cruise and sink' mode showing reduced rates of fluid deformation.

If the described swimming patterns of copepod species and their suggested potential diets are applied to the impacts found for the Hopavågen food web, the following picture emerges. *T. longicornis*, as a hydrodynamically 'loud' suspension feeder, may have lacked the ability of effectively capturing

heterotrophic ciliates and was consequently limited to the ingestion of less abundant diatoms. This practically exposed *T. longicornis* to starvation, resulting in an overall increase of its  $\delta^{15}\text{N}$ , independent of copepod abundance. In contrast, *C. hamatus* is hydrodynamically 'quieter' (Tiselius and Jonsson 1990) and may therefore readily approach fast swimming ciliates. Its  $\delta^{15}\text{N}$  increased most strongly of all copepod species with decreasing copepod densities, indicating that this species possibly exerted the highest grazing pressure on ciliates in the mesocosm bags. For both, *T. longicornis* and *C. hamatus*, diets suggested from their swimming and feeding behaviour would thus be in accordance with their isotopic patterns in the Hopavågen mesocosm.

The feeding behaviour of the third copepod species, *P. elongatus*, was described by Tiselius and Jonsson (1990) as adapted to the suspension feeding of small prey, comparable to the foraging behaviour of *T. longicornis*. However, the isotopic pattern in the mesocosm bags was similar to that of *C. hamatus*, not of *T. longicornis*, showing low, yet significant enrichment of its  $\delta^{15}\text{N}$  along the copepod density gradient. Possible reasons may be a higher efficiency in capturing smaller ciliates, or prey switching due to a change of its foraging mode. This phenomenon is well documented for the copepod *Acartia tonsa*. It may switch from suspension feeding on diatoms to ambush feeding on ciliates, when these are abundant (Kiørboe et al. 1996). Similarly, the copepod *Centropages typicus* may show a qualitative change in its feeding behaviour, swimming slow in a 'helical' mode in the presence of ciliates and preying more strongly on these (Capparo et al. 1998). To my knowledge, prey switching has not been described for *T. longicornis* or *P. elongatus*. Hence, one may only speculate about the capability of *P. elongatus* of changing its foraging mode in the absence or scarcity of immotile prey.

Different levels of nutritional stress experienced by the three copepod species, may seriously complicate the interpretation of the stable isotope patterns. Recently, Adams and Sterner (2000) demonstrated that a low relative nitrogen content in phytoplankton (high C:N ratio), and - in its extreme - the complete lack of food may increase the  $\delta^{15}\text{N}$  of freshwater zooplankton by  $\sim 0.2$  to  $0.4\text{‰}$  (Adams and Sterner 2000). This finding implies that zooplankton species with a high nitrogen demand may suffer more strongly from nutritional stress and, consequently, show a stronger increase in their  $\delta^{15}\text{N}$  than species with a lower nitrogen demand. Two observations indicate that *T. longicornis* was the species with the highest demand of nitrogen in the mesocosm bags. First, body nitrogen content was  $\sim 50\%$  higher for *T. longicornis* than *C. hamatus* (Table 10, Chapter V.3, no data for *P. elongatus*). And second, daily carbon requirements, and thus possibly also nitrogen requirements, are equally around double for *T. longicornis* than for *Centropages* or *Pseudocalanus* (Kiørboe et al. 1985). Therefore, it may be assumed that nutritional stress was especially high for *T. longicornis* during the experiment. This may be indicated in the relatively high overall enrichment of *T. longicornis*  $\delta^{15}\text{N}$  in all bags ( $\sim 0.4\text{‰}$ ). For *P. elongatus*, it was substantially lower in the highest density treatments ( $0.1\text{‰}$ ), where nutritional stress may be expected most pronounced. This notion further implies that *T. longicornis* would have to ingest proportionately more of an identical diet in order to 'experience' a

similar level of nutritional (nitrogen) stress as *P. elongatus*. Or, conversely, *T. longicornis* would have to feed far more intensely on ciliates in order to ‘show’ a similar increase of its  $\delta^{15}\text{N}$  signatures with copepod density as *P. elongatus*.

As a consequence, the interpretation of the isotopic patterns found for the copepod species would change dramatically. Changes of copepod  $\delta^{15}\text{N}$  along a density gradient would thus not reflect trophic enrichment due to diets differing in their contribution of heterotrophic prey as proposed in the model. Instead, they would reflect species-specific patterns of nitrogen demand or nutritional stress along a zooplankton density gradient. In this sense, the lack of correlation of *T. longicornis*  $\delta^{15}\text{N}$  would not indicate that this species was practically starving because it could not efficiently capture ciliates. Rather, *T. longicornis* would be experiencing high nutritional stress due to a high nitrogen demand, even if it was readily feeding on ciliates. *P. elongatus* and, especially, *C. hamatus* would experience less nutritional stress and consequently become more enriched in their  $\delta^{15}\text{N}$  as food becomes more available. Here again, one species does not fit the proposed explanation, as *C. hamatus* combines both, strongest enrichment along the density gradient, indicative of low nutritional stress, and a high enrichment in bags of the highest treatment densities (0.4‰), comparable to that of *T. longicornis*.

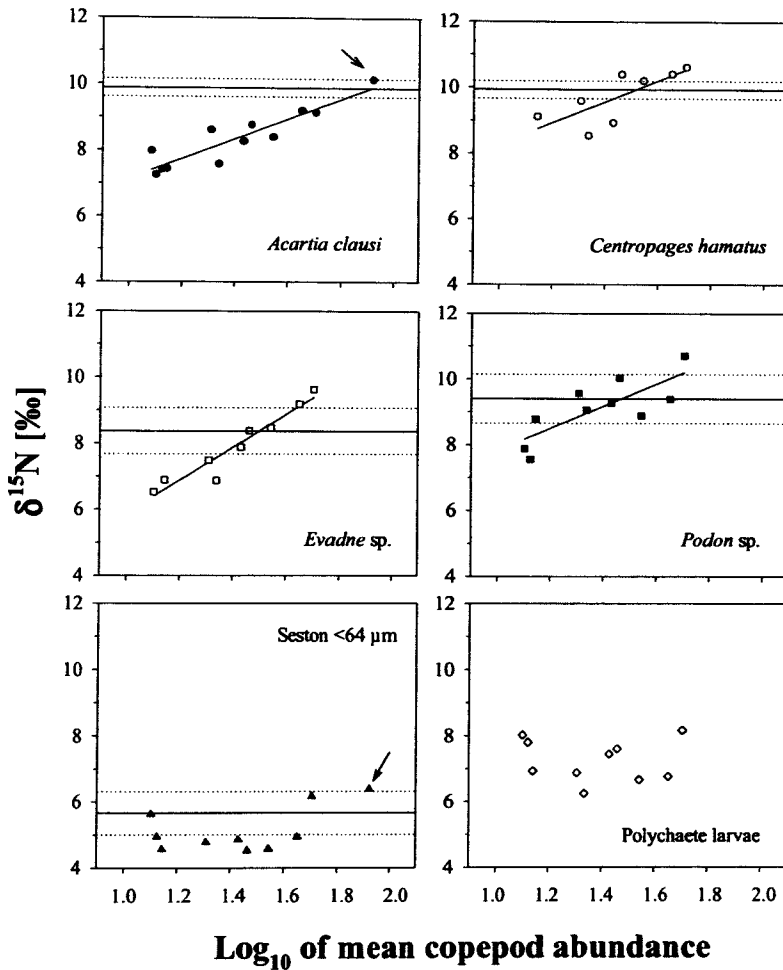
Therefore, I suggest a combination of both mechanisms to explain the patterns of copepod  $\delta^{15}\text{N}$ . The enrichment of the  $\delta^{15}\text{N}$  in bags of the highest density treatments over start values indicates that the nutritional stress was similarly high for *T. longicornis* and *C. hamatus* (~0.4‰) and low for *P. elongatus* (0.1‰). This presumably originates from differing levels of dietary nitrogen demand. Yet, while *T. longicornis* experienced a similar level of nutritional stress in all bags, irrespective of copepod density, *C. hamatus* was ‘capable’ of ‘reducing’ nutritional stress with decreasing copepod densities by actively grazing on particles. As a consequence, *C. hamatus* showed the strongest increase of its  $\delta^{15}\text{N}$  along the density gradient. This capability probably resulted from a more carnivorous, because less ‘noisy’ foraging mode compared to *T. longicornis*, permitting the capture of ciliates. For the copepod *P. elongatus*, enrichment of the  $\delta^{15}\text{N}$  by grazing was the ‘easiest’ to achieve, because its dietary nitrogen demand was lowest. While I still conclude that ciliates probably escaped predation by *T. longicornis*, and *C. hamatus* strongly grazed ciliates, it is not clear what the diet of *P. elongatus* was. This is because even low grazing on diatoms or ciliates would significantly increase its  $\delta^{15}\text{N}$  due to its seemingly low nitrogen demand.

#### **IV.5 Kiel Fjord $\delta^{15}\text{N}$**

##### **IV.5.1 Results**

Final  $\delta^{15}\text{N}$  of both, calanoid copepods and cladocerans were positively correlated with copepod densities (Figure 10). As the only zooplankton taxon, polychaete larvae showed no correlation of final  $\delta^{15}\text{N}$  with mean copepod densities. For *C. hamatus*, and the cladocerans *Podon* and *Evadne* the isotopic pattern was similar: Final  $\delta^{15}\text{N}$  were similar to start  $\delta^{15}\text{N}$  at medium copepod densities, yet increased or decreased at higher or lower copepod densities, respectively. In contrast, all  $\delta^{15}\text{N}$  of *A.*

*clausi* were lower than start  $\delta^{15}\text{N}$ , except for data from bag 24. The range of  $\delta^{15}\text{N}$  values was lowest for *C. hamatus* (up to 2.1‰) and relatively higher for *A. clausi* and the cladocerans *Podon* and *Evadne* (up to 2.9 and 3.1‰, respectively). The strongest decrease of final  $\delta^{15}\text{N}$  with respect to the mean initial  $\delta^{15}\text{N}$  was 2.5‰ for *A. clausi*, followed by 1.8 to 1.9‰ for *Evadne* and *Podon*, respectively.



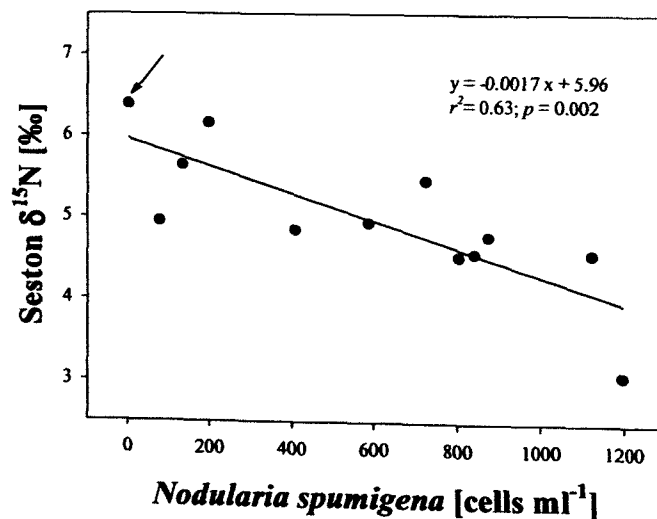
**Figure 10:** Final  $\delta^{15}\text{N}$  [‰] of zooplankton and seston  $<64\ \mu\text{m}$  as a function of  $\text{Log}_{10}$ -transformed mean copepod densities. The mean and  $\pm 1$  SD of start  $\delta^{15}\text{N}$  are indicated as solid and dotted horizontal lines, respectively. Replicate measurements of start values are pseudoreplicates of within-variability as they were taken from start populations in the barrel. No start data are available for polychaete larvae. The arrow indicates data from bag 24. Equations and statistics of linear regressions are given in Table 9.

Linear regressions fitted to the data show that *Evadne* was the zooplankton with the best fit of correlation and the highest slope value (Table 9). Similarly, *Podon* sp. had a high slope value, while that of copepods was less steep.

**Table 9:** Results of linear regression analyses of final zooplankton  $\delta^{15}\text{N}$  [‰] against  $\text{Log}_{10}$ -transformed mean copepod abundances shown in Figure 10. Mean copepod abundances were calculated as the arithmetic mean of copepod abundances on 4, 7, 10 and 13 September. Data from bag 24 on 13 September were not excluded from regression analysis (only *A. clausi*). Symbols are: \* ( $p < 0.05$ ), \*\*\* ( $p < 0.0005$ ).

taxon	linear regression	slope	$r^2$	$p$
<i>Acartia clausi</i>	$y = 3.00 x + 4.15$	3.00	0.84	***
<i>Centropages hamatus</i>	$y = 2.67 x + 5.89$	2.67	0.55	*
<i>Evadne</i> sp.	$y = 4.96 x + 0.93$	4.96	0.92	***
<i>Podon</i> sp.	$y = 3.17 x + 4.77$	3.17	0.62	***

Final seston  $\delta^{15}\text{N}$  were not correlated with zooplankton abundance (Figure 10), but were generally lower with respect to start  $\delta^{15}\text{N}$ . A correlation of final seston  $\delta^{15}\text{N}$  with *N. spumigena* cell concentrations showed that seston  $\delta^{15}\text{N}$  were significantly lower where *N. spumigena* concentrations were higher (Figure 11). Final seston  $\delta^{15}\text{N}$  values ranged from 3.1 to 6.3‰ (bag 24). The strongest decrease of final seston  $\delta^{15}\text{N}$  with respect to the mean initial  $\delta^{15}\text{N}$  was 2.5‰, similar to the decrease noted for *A. clausi* (2.5‰).



**Figure 11:** Final  $\delta^{15}\text{N}$  [‰] of seston as a function of *N. spumigena* concentrations on 13 September. A linear regression was fitted to the data. The arrow indicates data from bag 24.

#### IV.5.2 Discussion: Direct and indirect pathways of diazotrophic N transfer to zooplankton

Zooplankton play an important role in nitrogen cycling. They incorporate nitrogen in body tissue, produce faecal pellets and excrete ammonia. Such excreted ammonia is typically depleted by ~2.7‰ with respect to the catabolized substrate (Checkley and Miller 1989). Based on these observations, it has been suggested that the low  $\delta^{15}\text{N}$  of POM found in the upper water column of the oligotrophic ocean is caused by zooplankton grazing (Checkley and Entzeroth 1985, Checkley and Miller 1989). While isotopically 'heavy'  $^{15}\text{N}$  is exported to the deep in copepod faecal pellets, 'light'  $^{14}\text{N}$  is retained in upper waters as recycled ammonium. The isotopically depleted ammonium further fuels phytoplankton production and leads to an increasingly depleted  $\delta^{15}\text{N}$  of both POM and zooplankton (Mullin et al. 1984).

The experimental setup in this mesocosm, did not allow for the export of copepod faecal pellets. Assuming - for the moment - that nitrogen fixation by diazotrophs was negligible, shifts in the relative distribution of 'heavy'  $^{15}\text{N}$  between zooplankton and seston may occur due to trophic fractionation. Thus, if zooplankton accumulate 'heavy'  $^{15}\text{N}$  in their biomass and excrete isotopically 'light' ammonium, seston  $\delta^{15}\text{N}$  may be expected to become gradually decreased as zooplankton densities increase. The resulting relationship would be a negative correlation of seston  $\delta^{15}\text{N}$  with zooplankton abundance. Under non-limiting food conditions, the  $\delta^{15}\text{N}$  of zooplankton may increase uniformly in all bags. This is because an increased retention of  $^{15}\text{N}$  by a higher zooplankton population is proportionately distributed over the number of individuals, resulting in similar increases of zooplankton  $\delta^{15}\text{N}$ , irrespective of abundance.

The results show that seston  $\delta^{15}\text{N}$  in the mesocosm bags were not correlated with copepod densities. Instead, final seston  $\delta^{15}\text{N}$  were significantly lower in bags where *N. spumigena* had increased more strongly. This indicates that the development of *N. spumigena* and, consequently, the fixation of 'new', isotopically 'light' nitrogen had a much stronger impact on seston  $\delta^{15}\text{N}$  than (supposed) zooplankton trophic fractionation (and retention of 'heavy'  $^{15}\text{N}$ ) and recycling of 'light' ammonium. Equally, increases of zooplankton  $\delta^{15}\text{N}$  due to trophic fractionation were apparently strongly outweighed by the incorporation of 'new' nitrogen. At lowest zooplankton densities, all zooplankton - but polychaete larvae - showed decreases of their  $\delta^{15}\text{N}$  of 1.4 to 2.5‰ with respect to start  $\delta^{15}\text{N}$ . Only, where zooplankton densities were high, the incorporation of 'new' nitrogen did not outweigh trophic fractionation.

While the data demonstrate that diazotrophic nitrogen was transferred to zooplankton, the pathway is not clear. Principally, nitrogen originating from *N. spumigena* may have been transferred to zooplankton directly, by grazing on *N. spumigena*. The direct grazing impact of zooplankton can be principally confined to grazing by the calanoid copepod *A. clausi*, as it was the dominant zooplankton. Indeed, copepods of the genus *Acartia* have been recently shown to feed and reproduce successfully on *N. spumigena* (Koski et al. 2002). Offered as a dietary supplement, *N. spumigena* may even improve *Acartia* egg production (Schmidt and Jónasdóttir 1997). Yet, *Acartia* was also reported to

avoid *Nodularia* sp. (Engström et al. 2000). In the Kiel Fjord mesocosm experiment, evidence for a direct grazing impact of *A. clausi* on *N. spumigena* was equally ambiguous (Chapter III.4). At least on a long-term, *A. clausi* clearly had no impact on either cell concentrations (Table 7), or filament length of *N. spumigena* (Figure 5). Yet, notably, final  $\delta^{15}\text{N}$  of *A. clausi* - in contrast to the  $\delta^{15}\text{N}$  of the other zooplankton - were all lower than initial  $\delta^{15}\text{N}$  (except in bag 24) indicating that a direct transfer of cyanobacterial nitrogen to this species seems possible to some extent.

Instead, there are two observations that support the assumption of an indirect pathway in the (main) transfer of cyanobacterial nitrogen to zooplankton. First, final  $\delta^{15}\text{N}$  of zooplankton species were significantly correlated with mean copepod densities, while *N. spumigena* abundances were not correlated to the same gradient. This suggests that the transfer of cyanobacterial nitrogen to zooplankton was obviously strongly linked to a process of copepod grazing, other than the direct ingestion of *N. spumigena*. Second, the mere fact that zooplankton  $\delta^{15}\text{N}$  were linearly correlated with copepod abundance indicates that the transfer of diazotrophic nitrogen to zooplankton occurred via dietary particles that were limiting zooplankton grazing (Figure 7). An unlimited supply of diazotrophic nitrogen, also at highest copepod treatment densities, would produce equally decreased zooplankton  $\delta^{15}\text{N}$  in all bags. As *N. spumigena* concentrations did not become decreased over time or along the density gradient, it may be assumed that they were not (substantially) directly grazed and, hence, not a limiting food source for zooplankton. This fact, however, applies for large ciliates and rotifers which were negatively affected by copepods (along the density gradient) and diminished in abundance to practical absence by the end of the experiment in all bags (Table 7).

Thus, one possible indirect pathway may be the transfer of diazotrophic nitrogen via a microbial trophic link. Trichomes of *N. spumigena* may support a diverse epicoenotic community, including bacteria, fungi and heterotrophic microzooplankton (Hoppe 1981). In the Kiel Fjord study, zooplankton (*A. clausi*) had a significantly negative impact on large ciliates, the rotifer *Synchaeta* sp., and on some potentially mixotrophic dinoflagellates (Table 7). Therefore, the trophic transfer of isotopically 'light' nitrogen to zooplankton via a microbial link 'bacteria-flagellates-ciliates/rotifers/heterotrophic dinoflagellates-zooplankton' (Arndt 1993) seems plausible. This would explain why final zooplankton  $\delta^{15}\text{N}$  decreased as a function of zooplankton densities, as particles 'mediating' diazotrophic transfer (ciliates, rotifers) were a limiting dietary resource.

An alternative or, to a certain extent, additional pathway may be nitrogen regeneration and loss of intracellular organic compounds due to 'sloppy feeding' by *A. clausi* (short-term impact on *N. spumigena*) and microzooplankton. Diazotrophic nitrogen may thus enter other plankton, such as diatoms, after the incorporation of recycled ammonia originating from *N. spumigena* nitrogen. If such dietary particles are subsequently ingested by zooplankton (e.g. *D. brightwellii*, Table 7), diazotrophic nitrogen would reach zooplankton via the link *N. spumigena* epicoenosis-ammonia (recycling)-dietary particles of zooplankton. Clearly, transfer of diazotrophic nitrogen via nutrient regeneration would thus reach all members of the food web, rapidly and nearly simultaneously.

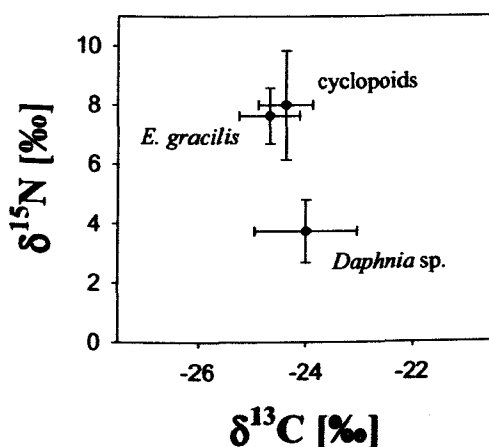
In tropical waters of the Pacific and Atlantic Ocean,  $N_2$  fixation by the filamentous diazotrophic cyanobacterium *Trichodesmium* spp. constitutes a major contribution to the nitrogen budget (Montoya et al. 2002). This finding may also hold true for summer conditions in the Baltic Sea. The results clearly demonstrate that diazotrophic nitrogen enters zooplankton rapidly, causing maximum decreases in zooplankton  $\delta^{15}N$  of 1.8 to 2.5‰ within only 9 days, similar to the maximum decrease of seston  $\delta^{15}N$  (2.5‰). The main transfer of diazotrophic nitrogen to zooplankton, however, seems not to be direct. The decoupling of decreases in zooplankton  $\delta^{15}N$  from decreases in POM  $\delta^{15}N$ , yet the strong correlation with zooplankton abundance indicates that diazotrophic nitrogen must have entered zooplankton indirectly via grazing on intermediate, heterotrophic microplankton in combination with nutrient regeneration. The apparent ‘speed’ at which this transfer to zooplankton takes place is likely to depend on interspecific differences of population dynamics. Steeper slopes for cladocerans may indicate a seemingly more rapid incorporation of diazotrophic nitrogen for these zooplankton than for copepods due to their parthenogenetic and therefore more rapid population turnover.

#### IV.6 Schöhsee $\delta^{15}N$

By the end of the Schöhsee experiment, *Daphnia* constituted a dominant portion of total zooplankton in the copepod treatments. Cyclopoid copepods had increased moderately. Therefore, isotopic samples could be obtained for calanoid copepods, cladocerans, and - in a few bags - also for cyclopoid copepods in the copepod treatment. Important variables (start and seston  $\delta^{15}N$ ) were not measured in this mesocosm study. Hence, conclusions were drawn from the findings of the other marine studies.

##### IV.6.1 Results

Combining data from all enclosure bags of the copepod treatment, the overall  $\delta^{15}N$  of the calanoid *Eudiaptomus gracilis* was within the same range as the mean  $\delta^{15}N$  of cyclopoid copepods (~8.0‰; Figure 12). Both calanoid and cyclopoid  $\delta^{15}N$  were significantly enriched by ~4‰ with respect to the  $\delta^{15}N$  of the cladoceran *Daphnia* sp. ( $p < 0.001$ , Tukey’s HSD test for unequal sample sizes).

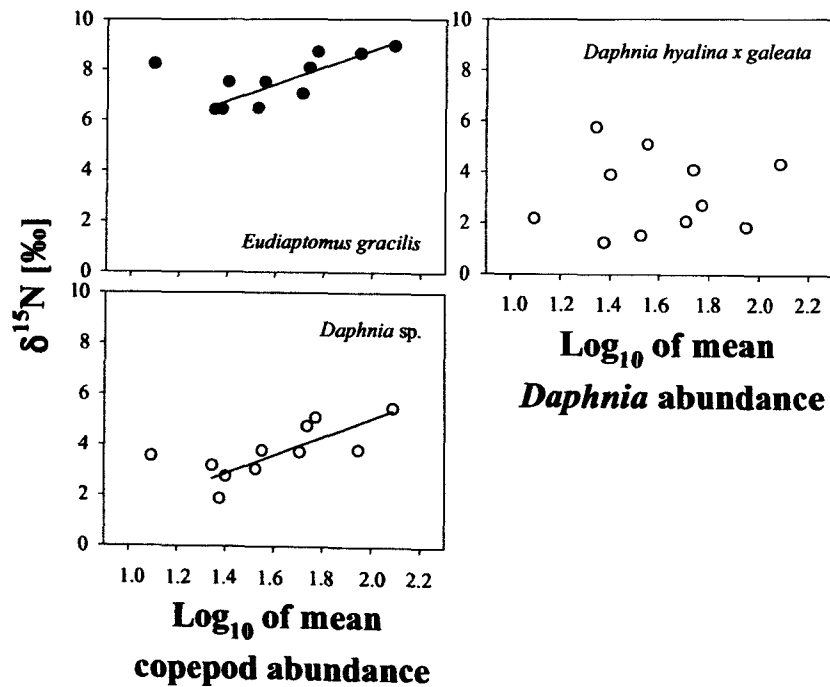


**Figure 12:** Final  $\delta^{15}N$  of *Daphnia*, cyclopoid copepods and *E. gracilis* in the copepod treatment. Symbols are means of 11 (*Daphnia* and *E. gracilis*, respectively) or 7 (cyclopoids) replicates. Samples of cyclopoid copepods consisted of a mix of different species and developmental stages. Bars represent  $\pm 1$  SD. *Daphnia*  $\delta^{15}N$  is significantly different from both, the  $\delta^{15}N$  of *E. gracilis* and of cyclopoid copepods ( $p < 0.001$ , respectively, Tukey’s HSD test for unequal sample sizes).



The comparably higher variability of the  $\delta^{15}\text{N}$  of cyclopoid copepods was probably due to the mixed assemblage of different developmental stages (copepodites and adults) and different species of cyclopoids.

Similar to the Kiel Fjord experiment, final  $\delta^{15}\text{N}$  of both, *E. gracilis* and *Daphnia* were significantly positively correlated with  $\text{Log}_{10}$ -transformed mean copepod densities in the copepod treatment (Figure 13). Zooplankton  $\delta^{15}\text{N}$  of the lowest copepod treatment density (5 ind  $\text{L}^{-1}$ ) was excluded from regression analysis (see below). The slope  $k$  of the linear regression was slightly steeper for the cladoceran *Daphnia* ( $k = 3.6$ ) than for the copepod *E. gracilis* ( $k = 3.4$ ). In contrast, no correlation was found for the final  $\delta^{15}\text{N}$  of the laboratory-reared *D. hyalina x galeata* in the cladoceran treatments.

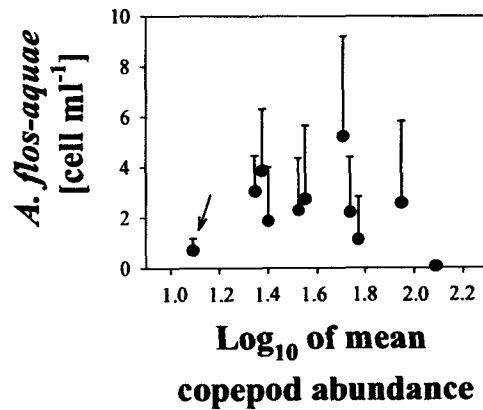


**Figure 13:** Final  $\delta^{15}\text{N}$  [‰] of zooplankton as a function of  $\text{Log}_{10}$ -transformed mean densities of copepods in the copepod treatment (left) and in the cladoceran treatment (right). Linear regressions are:  $y = 2.03 + 3.37x$ ;  $r^2 = 0.73$ ,  $p < 0.005$  (*E. gracilis*), and  $y = -2.24 + 3.64x$ ;  $r^2 = 0.67$ ,  $p < 0.005$  (*Daphnia* sp.). Data of the lowest copepod treatment density (5 ind  $\text{L}^{-1}$ ) were excluded from regressions.

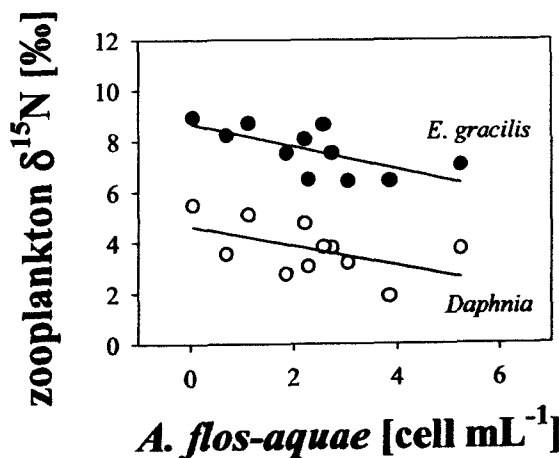
As in the Kiel Fjord study, a filamentous diazotrophic cyanobacterium (*Anabaena flos-aquae*) was present in the enclosure bags (Figure 14). The analysis in Table 4 showed that copepods had a significantly negative impact on *A. flos-aquae* concentrations in this experiment, indicating that some portion of direct incorporation of diazotrophic nitrogen, at least by copepods, occurred. Notably, *A.*

*flos-aquae* was practically absent in the lowest treatment density bag (5 ind L<sup>-1</sup>), where the  $\delta^{15}\text{N}$  of both *E. gracilis* and *Daphnia* were relatively high, and did not 'fit' to the general decrease of zooplankton  $\delta^{15}\text{N}$  with decreasing zooplankton densities (Figure 13). Concentrations of *A. flos-aquae* were similarly low in one bag of the highest density treatment. Yet, if we expected the  $\delta^{15}\text{N}$  to change marginally at high zooplankton densities, but strongly at low densities (Figure 7), low concentrations of diazotrophic cyanobacteria may significantly affect zooplankton  $\delta^{15}\text{N}$  only at low zooplankton densities.

**Figure 14:** Mean concentrations (17, 21, 24 and 28 August) of the cyanobacterium *Anabaena flos-aquae* in the copepod treatments. Bars represent 1 SD, for clarity shown in only one direction. The arrow points to the lowest copepod treatment density (5 ind L<sup>-1</sup>).



A linear correlation of zooplankton  $\delta^{15}\text{N}$  and mean concentrations of *A. flos-aquae* significantly explained some of the variability (47%) of *E. gracilis*  $\delta^{15}\text{N}$  (Figure 14). *Daphnia*  $\delta^{15}\text{N}$  was correlated with *A. flos-aquae* concentrations at  $p < 0.1$ .



**Figure 15:** Final  $\delta^{15}\text{N}$  [‰] of zooplankton as a function of *A. flos-aquae* cell concentrations in the copepod treatment. Linear regressions are:  $y = 8.7 - 0.4x$ ;  $r^2 = 0.47$ ,  $p < 0.05$  (*E. gracilis*), and  $y = 4.6 - 0.4x$ ;  $r^2 = 0.28$ ,  $p < 0.1$  (*Daphnia* sp.).

#### IV.6.2 Discussion: The trophic position of *Daphnia* and *Eudiaptomus*

In the Schöhsee experiment, final  $\delta^{15}\text{N}$  of calanoid copepods and cladocerans were separated by  $\sim 4\text{‰}$ . This is approximately the isotopic difference proposed to correspond to one-trophic level difference (Minagawa and Wada 1984). Considering that ethanol fixation may increase the  $\delta^{15}\text{N}$  only negligibly for cladocerans, yet by up to  $1.9\text{‰}$  for copepods (Table 8), there still remains a relatively large ( $\sim 2\text{‰}$ ) difference between the final  $\delta^{15}\text{N}$  of copepods and cladocerans. This indicates that the degree of herbivory between calanoid copepods and *Daphnia* may be indeed large, and that the calanoid *E. gracilis* isotopically resembles carnivorous cyclopoid copepods more closely than *Daphnia*.

There is a series of studies showing that the  $\delta^{15}\text{N}$  of herbivorous cladocerans (mostly daphnids) and co-occurring calanoid copepods (diaptomids) generally differ by  $\sim 2.5$  to  $3.8\text{‰}$  (Gu et al. 1994, Yoshioka et al. 1994, Meili et al. 1996, Jones et al. 1998). Also, freshwater calanoids and cyclopoids have been found to have similarly close isotopic signatures (Kling et al. 1992, Yoshioka et al. 1994, Yoshii et al. 1999). Therefore, the isotopic difference between *Daphnia* and *E. gracilis* calculated in our study ( $3.2$  to  $4.8\text{‰}$ , or corrected for ethanol fixation:  $1.2$  to  $2.8\text{‰}$ ) supports these findings and demonstrates that herbivorous cladocerans and calanoid copepods may be approximately one trophic level apart ( $3.4\text{‰}$  average distance between trophic levels: Minagawa and Wada 1984).

This difference probably stems from differences in their nutrition. Both *Daphnia* (Jürgens 1994) and *E. gracilis* (Ehret 2000) have been shown to feed on heterotrophic protozoa. Due to mechanical restrictions imposed by its filtering apparatus, large ( $>30$  to  $50\text{ }\mu\text{m}$ ) ciliates escape predation by *Daphnia*. Calanoid copepods in general, and *E. gracilis* specifically, have been shown to select for large ciliates in many freshwater studies (Wiackowski et al. 1994, Burns and Schallenburg 1996, Adrian and Schneider-Olt 1999, Ehret 2000). Hence, when feeding 'higher up' the microbial food chain on larger ciliates, calanoid copepods seem to be ingesting a sufficient large amount of isotopically 'heavy'  $^{15}\text{N}$ , so as to exhibit  $\delta^{15}\text{N}$  values as far as one trophic level above *Daphnia* and close to predatory cyclopoid copepods.

A positive correlation of final zooplankton  $\delta^{15}\text{N}$  with copepod abundance was found in the copepod treatments, resembling those found for zooplankton  $\delta^{15}\text{N}$  in the Kiel Fjord experiment. From the presence of the diazotrophic cyanobacterium *A. flos-aquae*, it may be assumed that 'new', atmospherically derived nitrogen was entering the planktonic food web. As proposed previously, indirect pathways via ingestion of intermediate heterotrophs and/or nutrient regeneration may result in a positive relationship between zooplankton  $\delta^{15}\text{N}$  and zooplankton abundance. The close-to absence of *A. flos-aquae* in the lowest treatment density may be the reason why zooplankton  $\delta^{15}\text{N}$  was not as decreased there as in bags of similarly low zooplankton densities. Also, some diazotrophic nitrogen may have been transferred directly to zooplankton, as copepods had a strong negative impact on *A. flos-aquae* (Table 4). Additionally, the  $\delta^{15}\text{N}$  of *E. gracilis* were significantly correlated with cell concentrations of *A. flos-aquae*, indicating an increased transfer of diazotrophic nitrogen to copepods at higher concentrations of *A. flos-aquae*. The steeper slope for the cladoceran *Daphnia* with respect to

*E. gracilis* may be the result of different population dynamics, as population turnover is higher in cladocerans than in copepods due to parthenogenetic reproduction. This was similarly observed in the Kiel Fjord experiment.

The lack of correlation of *Daphnia*  $\delta^{15}\text{N}$  with zooplankton density in the cladoceran treatment may not be surprising as individuals were reared in several batches in the laboratory, and, thus, probably had very different start  $\delta^{15}\text{N}$ . In contrast, zooplankton of the copepod treatment originated from the lake and were homogeneously mixed in barrels before adding them to the mesocosm bags.

#### IV.7 Summary and conclusions

The rationale for the analysis of zooplankton  $\delta^{15}\text{N}$  was to determine interspecific differences in assimilated diets of zooplankton. This is because stable isotope signatures may be seen as “actual assimilation integrated over the time scale of tissue turnover in the organism” (Kling et al. 1992). With this, the analysis of stable isotopes differs from insights gained from gut content analysis or incubation experiments, because they measure assimilation of elements, not ingestion of particles. Therefore, the analysis of stable isotope signatures may provide additional, perhaps complementary information to the analysis of particle grazing.

The predictions of trophic enrichment along a zooplankton density gradient (Figure 7) could be applied to the Hopavågen mesocosm experiment only, but not to the other studies. This is because the activity of diazotrophic cyanobacteria of importing ‘new’, isotopically ‘light’ atmospheric nitrogen and its subsequent transfer to zooplankton proved to outweigh trophic enrichment of zooplankton  $\delta^{15}\text{N}$ . The interpretation of species-specific isotopic patterns was nevertheless difficult despite a simple food web structure of potentially edible particles (diatoms and ciliates), and the fact that the zooplankton (copepods) were similar with respect to size (~1 mm) and life history (in contrast, e.g. to parthenogenetic cladocerans). It was shown that even presumably small differences in starvation tolerance or nitrogen demand may substantially hamper the interpretation of zooplankton  $\delta^{15}\text{N}$ .

Despite this, some conclusions may be drawn. First, interspecific differences seem large (~4‰) between different freshwater taxa, accounting for an up to one-trophic level difference. Probably due to selective particle discrimination, the calanoid copepod *E. gracilis* was as enriched in its  $\delta^{15}\text{N}$  as predatory cyclopoid copepods. Judging from its isotopic signal, the freshwater cladoceran *Daphnia* seemed to be the more herbivorous zooplankton. In contrast, differences between marine zooplankton  $\delta^{15}\text{N}$  were generally less pronounced (~2‰). The appendicularian *O. dioica* fell isotopically within the range of omnivorous copepods. This suggests that *O. dioica* may ingest an approximately equal proportion of heterotrophic prey (heterotrophic nanoflagellates) as omnivorous copepods (ciliates). Second, there seem to be differences in carnivory by similarly sized copepods, as *C. hamatus* was probably feeding more strongly on ciliates than *T. longicornis* due to its less ‘noisy’ foraging behaviour. These differences may arise from the different foraging strategies of calanoid copepods. Third, ‘new’ nitrogen from diazotrophic cyanobacteria enters both, freshwater and marine zooplankton

rapidly. While direct ingestion may contribute to the decrease of zooplankton  $\delta^{15}\text{N}$ , the positive linear relationship of zooplankton  $\delta^{15}\text{N}$  with zooplankton density indicates that indirect transfer via the ingestion of intermediary microplankton (ciliates, rotifers) combined with nutrient regeneration, is probably just as if not more important. Fourth, diazotrophic nitrogen is seemingly more rapidly incorporated into populations of cladocerans than copepods because of faster population turnover.

## V Testing ecological stoichiometry

### V.1 Introduction

Ecological stoichiometry asserts that the relative proportion of elements in primary producers and their consumers may be a critical factor in structuring food webs (Sternern 1990, Elser and Urabe 1999). This theoretical framework implies that the elemental composition of primary producers may inhibit somatic and reproductive growth of consumers, if they are deficient in an element required by consumers (bottom-up effect). Equally, it predicts that the growth of primary producers may become limited by consumers, because these retain element(s) in their biomass according to their own stoichiometric demands and release those of minor importance in excess (top-down effect). Thus, the theory of ecological stoichiometry differs from the classical view which sees grazing as a mechanism of reducing nutrient stress because herbivores fertilise (the ungrazed or surviving) algae by regenerating nutrients (Sternern 1986).

In the past decade, stoichiometric considerations have prompted numerous studies, especially in pelagic freshwater systems. Two aspects seem to make freshwater systems ideal for stoichiometric studies. First, phytoplankton growth in freshwater is often severely limited by P (Hecky et al. 1993). Consequently, elemental ratios of suspended particulate organic matter (POM) in lakes are often strongly elevated ( $C:P > 300:1$ , Hecky et al. 1993) over the Redfield ratio ( $C:N:P = 106:16:1$ , Redfield 1958), which allows for optimal cell growth of phytoplankton (Goldman et al. 1979). Second, zooplankton stoichiometry has been found to be fairly stable within species (Hessen and Lyche 1991) and relatively independent of food supply or its chemical composition (Andersen and Hessen 1991). Thus, zooplankton stoichiometry contrasts markedly with the high variability of elemental ratios found for POM in freshwater (Hecky et al. 1993). The frequent freshwater cladoceran *Daphnia* combines characteristics that are favourable for experimentation. Individuals are not only rich in P ( $>1\%$  of dry weight, Hessen 1990) but also generally quite large ( $>1$  mm). Consequently, *Daphnia* is characterised by both, a pronounced elemental imbalance (the difference between food and consumer elemental ratio) and high specific P requirements on an absolute scale.

In the marine environment,  $C:N:P$  ratios of suspended POM are relatively uniform and fairly close to the Redfield ratio (Redfield 1958, Copin-Montegut and Copin-Montegut 1983). Marine zooplankton may contain relatively more N and, especially cladocerans, more P than suspended POM (Gismervik 1997). However, the small elemental imbalance between zooplankton and POM suggests stoichiometric effects to be weak (Walve and Larsson 1999).

In the mesocosm studies, I tested one of the predictions of ecological stoichiometry, namely that zooplankton may act both as a sink and source of elements (N or P, respectively) depending on their own and resource stoichiometry (Sternern 1990). The element in shortest supply for zooplankton is thereby retained in zooplankton biomass, resulting in phytoplankton being drained from this element, whereas the element in excess is preferentially released in order to maintain stoichiometric balance.

(Urabe et al. 1995). Therefore, P-rich zooplankton (freshwater *Daphnia* and marine appendicularians) were expected to increase C:P ratios of seston (used as a surrogate for phytoplankton), while N-rich zooplankton (marine and freshwater copepods) would increase seston C:N ratios, if based on an initially balanced Redfield stoichiometry (C:N:P = 106:16:1).

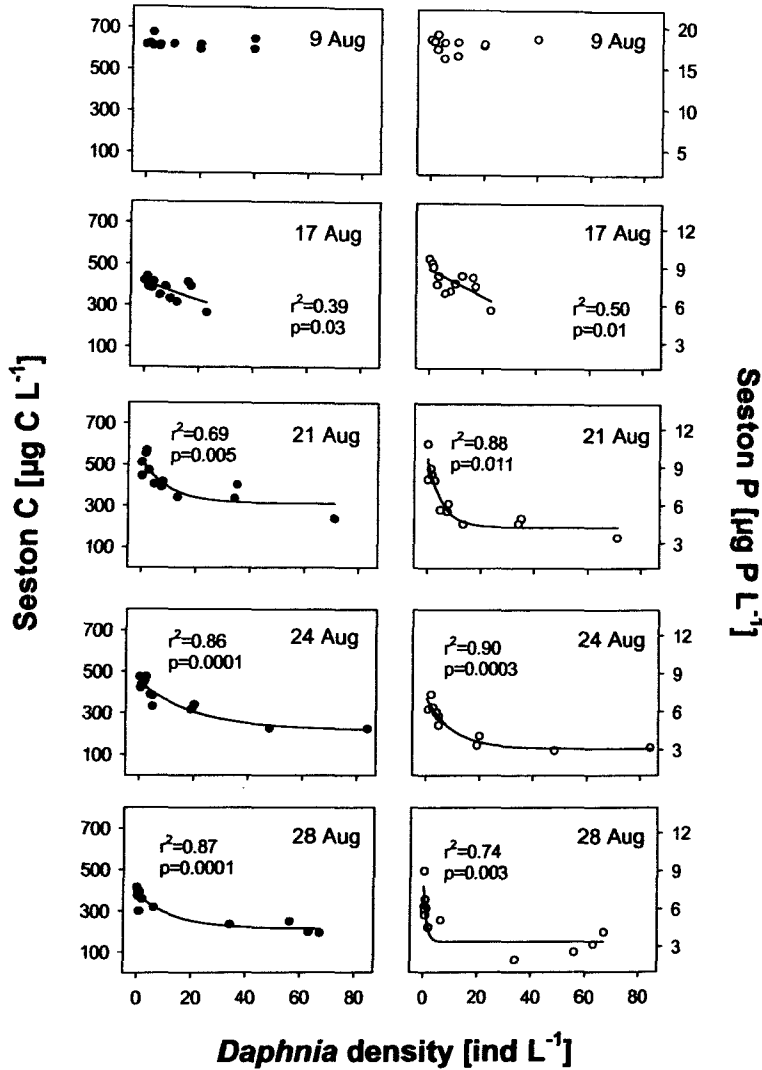
### V.2.1 Schöhsee stoichiometry

In the Schöhsee experiment, bags of the copepod treatment became severely contaminated by *Daphnia* towards the end of the experiment, precluding the analysis of stoichiometric effects for freshwater copepods. Therefore, only data for the *Daphnia* treatment are shown. Moreover, significant stoichiometric effects were only found for P, which is why no data are shown for seston N or seston C:N ratios. Here, and later in the discussion, I consequently focus on the stoichiometric effects of *Daphnia* on the P content of its diet.

The experimental setup in this first mesocosm experiment (a tube to remove settled organic matter from the bottom of the bags, see Chapter II.2) allowed to test which of two proposed mechanisms may act as a greater P drain for phytoplankton: (1) the retention of elements in *Daphnia* biomass (Elser and Hassett 1994), or (2) the sedimentation of P bound in exoskeletal remains after *Daphnia* moulting (Vrede et al. 1999). P associated with the carapace of *Daphnia*, may constitute ~14% of total body P (Vrede et al. 1999), and hence represent a potentially strong mechanism of P removal. (Similar sedimentation effects for marine copepods could not be accounted for in later experiments, because the great loss of zooplankton due to the removal of 'sediment water' via the tube required to refrain from this setup).

### V.2.1 Results

**Seston** - Initial values of seston C and seston P ranged from 600 to 650  $\mu\text{g C L}^{-1}$  and 16 to 18  $\mu\text{g P L}^{-1}$  on 9 August (Figure 16). Both seston C and P content dropped successively during the course of the experiment. Lowest values of seston C (~200 to 250  $\mu\text{g C L}^{-1}$ ) and seston P (~2 to 3  $\mu\text{g P L}^{-1}$ ) were observed in bags of *Daphnia* densities >30 ind  $\text{L}^{-1}$  on 24 and 28 August. On sampling days from 17 August onwards, significant negative correlations with *Daphnia* densities were evident for both, seston C and seston P. Equations of regressions on data of 21, 24 and 28 August suggest that seston P was reduced to a residual value of ~3 to 4  $\mu\text{g P L}^{-1}$  with increasing *Daphnia* densities. The difference of seston P content in control bags and bags of the highest *Daphnia* densities was on average  $6.3 \pm 1.4 \mu\text{g P L}^{-1}$  (calculated from data of 21, 24 and 28 August).

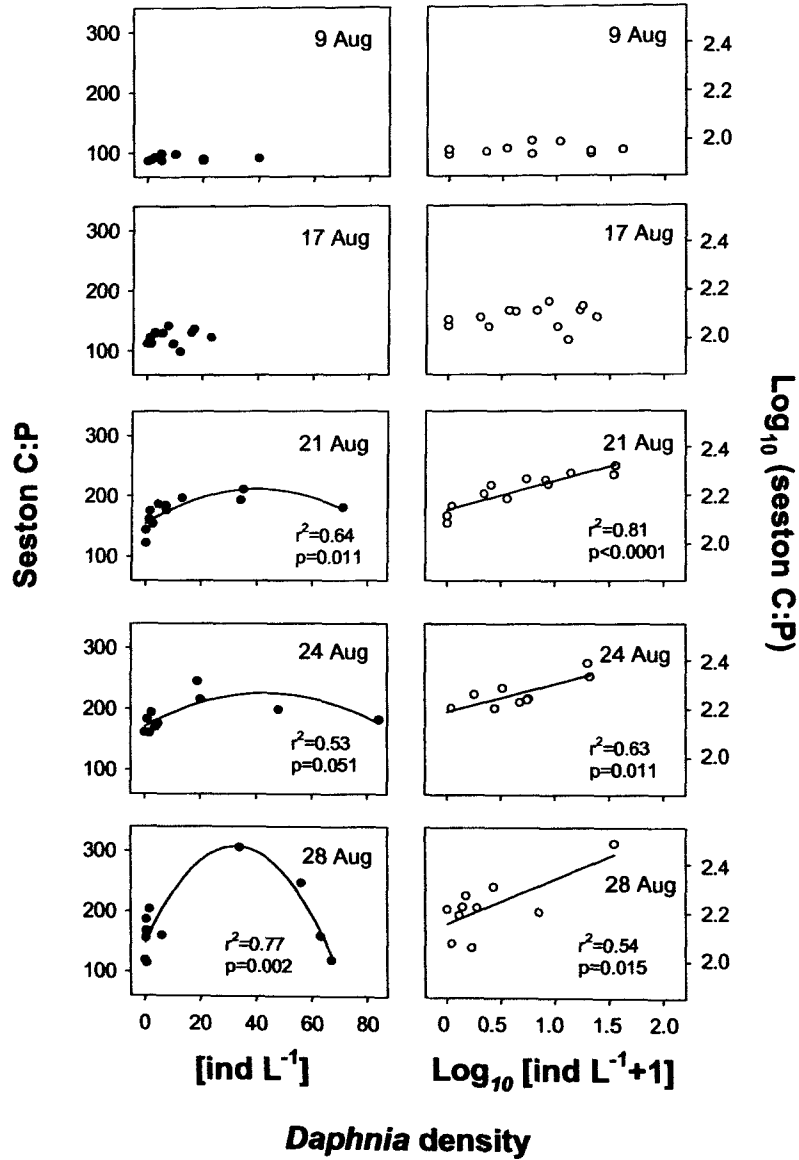


**Figure 16:** Seston C (left) and seston P (right) against *Daphnia* densities. Linear regressions on 17 August are:  $y=410-4.3x$  (Seston C) and  $y=8.8-0.1x$  (Seston P). Exponential regressions fitted to seston C are:  $y=311+208e^{-0.1x}$  (21 August),  $y=218+231e^{-0.05x}$  (24 August) and  $y=211+170e^{-0.07x}$  (28 August). Exponential regressions fitted to seston P are:  $y=4.3+5.4e^{-0.19x}$  (21 August),  $y=3.1+4.0e^{-0.10x}$  (24 August) and  $y=3.4+4.8e^{-0.94x}$  (28 August).

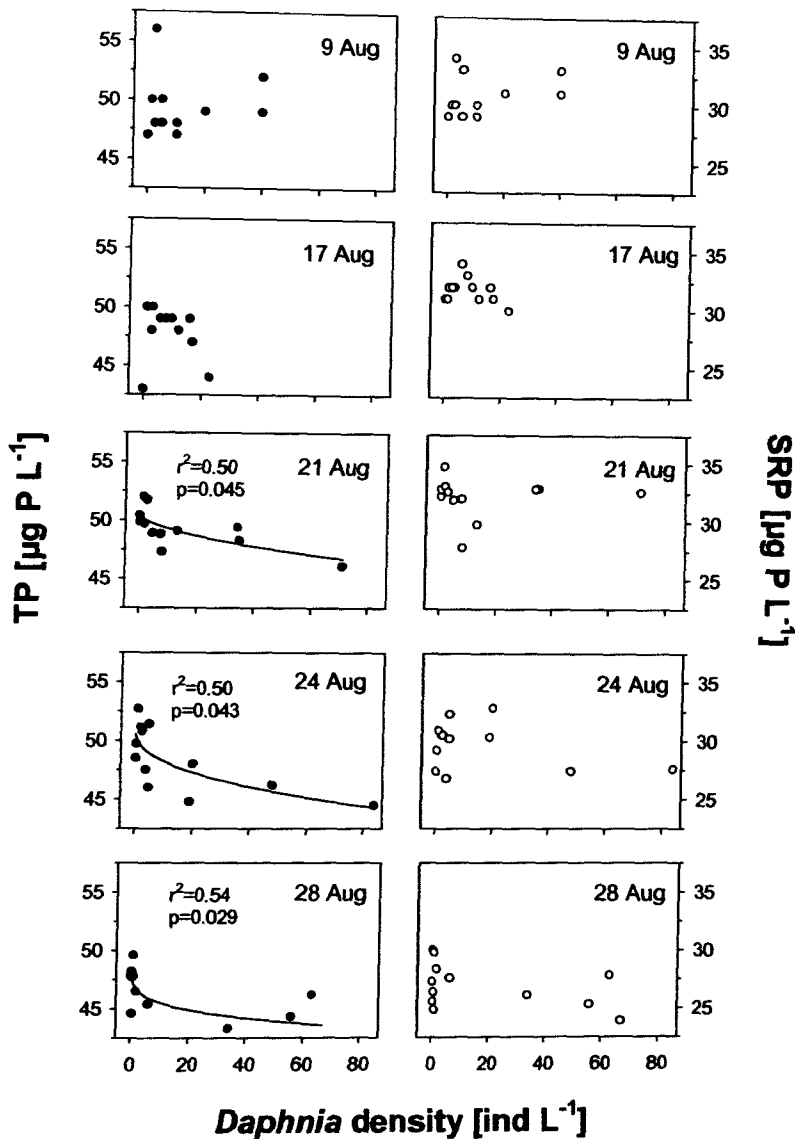
Seston C:P ratios on 9 August were around the Redfield ratio of C:P = 106:1 (range: 80:1 to 116:1), indicative of P sufficiency for phytoplankton growth (Goldman et al. 1979) (Figure 17). On 17 August seston C:P ratios were generally higher, yet <180:1. Seston C:P ratios were correlated to *Daphnia* densities by a second-order polynomial function on 21, 24 and 28 August. Highest seston C:P values (max. C:P = 306:1) were found in bags with *Daphnia* densities of ~30 to 50 ind L<sup>-1</sup>. Logarithmic



transformation of seston C:P ratios and *Daphnia* abundances  $\leq 40$  ind  $L^{-1}$  revealed significant positive linear relationships for 21, 24 and 28 August.



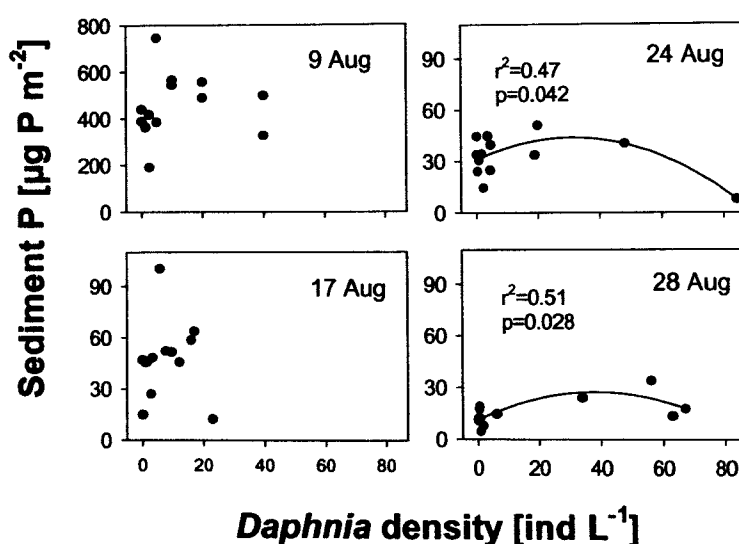
**Figure 17:** Seston C:P ratios against *Daphnia* densities (left) and Log-converted ( $\text{Log}_{10}+1$ ) seston C:P ratios against Log-converted ( $\text{Log}_{10}+1$ ) *Daphnia* densities (right). For Log-converted data (right) only *Daphnia* densities  $\leq 40$  ind  $L^{-1}$  were included. Polynomial second-order functions (left) are:  $y=154+2.9x-0.04x^2$  (21 August),  $y=171+2.6x-0.03x^2$  (24 August) and  $y=147+9.8x-0.15x^2$  (28 August). Linear regressions (right) are:  $y=2.2-0.05x$  (21 August),  $y=2.2-0.06x$  (24 August) and  $y=2.2-0.13x$  (28 August).



**Figure 18:** TP (left) and SRP (right) against *Daphnia* densities. Regressions fitted to TP in cladoceran treatments are:  $y=51-0.35x^{0.56}$  (21 August),  $y=51-1.03x^{0.42}$  (24 August) and  $y=50-2.46x^{0.21}$  (28 August).

**TP & SRP** - Initial values of TP (total P) and SRP (soluble reactive P) on 9 August ranged from 47 to 56  $\mu\text{g P L}^{-1}$  and from 29 to 34  $\mu\text{g P L}^{-1}$ , respectively (Figure 18). As for seston P, TP was significantly negatively correlated with *Daphnia* densities on 21, 24 and 28 August. Significant positive linear correlations between seston P and TP values on 21 ( $r^2 = 0.47$ ,  $p=0.01$ ), 24 ( $r^2 = 0.61$ ,  $p=0.005$ ) and 28 August ( $r^2 = 0.43$ ,  $p=0.015$ ) indicate that the decrease of TP with increasing *Daphnia* densities on

these days was due to the reduction of seston P within TP (not shown). Moreover, the difference of TP values in control bags and bags of the highest *Daphnia* densities was on average  $5.2 \pm 1.0 \mu\text{g P L}^{-1}$  (calculated from data of 21, 24 and 28 August), which corresponds quantitatively to the decrease of particulate P in the seston fraction ( $6.3 \pm 1.4 \mu\text{g P L}^{-1}$ ; Figure 16). The inorganic P with which the mesocosm bags had been fertilised at the beginning of the experiment ( $106 \text{ mg P bag}^{-1}$ , see Chapter II.2) was not substantially absorbed by phytoplankton during the course of the experiment. Instead, SRP concentrations corresponded well with the added amount of inorganic P in all bags at approximately the same quantity as added ( $\sim 25$  to  $35 \mu\text{g P L}^{-1}$ ), and showed no correlation with *Daphnia* density on any sampling day.



**Figure 19:** Sediment P against *Daphnia* densities. Polynomial second-order functions are:  $y=31+0.8x-0.01x^2$  (24 August) and  $y=11+0.9x-0.01x^2$  (28 August).

**Sediment** - On 9 August, shortly before adding *Daphnia*, the range of P in settled organic particulate matter (hereafter called sediment P) was high ( $190$  to  $745 \mu\text{g P m}^{-2}$ ; Figure 19). Later on, sediment P was  $<100 \mu\text{g P m}^{-2}$  in all bags and sampling days, but usually it was  $<60 \mu\text{g P m}^{-2}$ . The amount of P removed by pumping off 'sediment water' ( $\sim 5 \text{ L}$ ), thus never exceeded 1% of the total amount of P bound in particulate matter (seston) of the overlying water column on 17, 24 and 28 August (not shown). As for seston data, a polynomial second-order function was fitted to the data, suggesting significant, though weak ( $0.02 < p < 0.05$ ), correlations between the amount of sediment P and *Daphnia* densities on 24 and 28 August. As sediment samples were not pre-screened, entire (and even live) animals were found on the filters. On 17 and 24 August, no correlations between the number of *Daphnia* per filter ( $<10 \text{ ind Filter}^{-1}$ ) and sediment P were found (not shown). On 28 August, however,

many entire *Daphnia* individuals (up to 49 ind Filter<sup>-1</sup>) were counted on the filters, resulting in a strong correlation between the number of *Daphnia* on the filter and the amount of P per filter ( $r^2 = 0.81$ ,  $p < 0.005$ ). This suggests that at least some portion of P measured on the filters must be attributed to the measurement of entire individuals rather than sedimented carapaces after moulting.

### V.2.2 Discussion: *Daphnia* population growth as a phosphorous drain for phytoplankton

Zooplankton-associated sedimentation processes can be basically summarised in the sinking of faecal pellets, of eggs (if produced), of dead individuals and of their moulting products. It is known for *Daphnia* that cladoceran faecal pellets disrupt to flocculent particles after excretion (Peters 1987), and that they are therefore probably quickly recycled within the epilimnion (Andersen 1997). P associated with the carapace of the cladoceran *Daphnia* may constitute around 14% of total body P (Vrede et al. 1999). Therefore, frequent moulting associated with rapid growth and subsequent sedimentation of moulting products has been proposed to represent a substantial P drain for the water column (Vrede et al. 1999).

This notion is, however, not supported by the data. On the contrary, it seems that either P is effectively removed by *Daphnia* prior to moulting or that, more probably, carapace-associated P is rapidly lost or remineralised. Resorption of carapace-bound P prior to moulting has, to my knowledge, not been quantified. Yet, Alstad et al. (1999) have shown that only 10% or less of the total amount of calcium in *Daphnia magna* was withdrawn prior to moulting, while about 50% was lost to the surrounding water. Given that calcium in the crustacean cuticle is principally bound as  $\text{CaCO}_3$  and  $\text{Ca}_3(\text{PO}_4)_2$  (Stevenson 1985), the resorption of carapace-associated P [bound as  $\text{Ca}_3(\text{PO}_4)_2$ ] by *Daphnia* might be as inefficient as it is for calcium located in the carapace. Hence, most of the P associated with the carapace may either be directly lost to the water column together with calcium, or remain bound to the carapace. Since the experimental design did not allow for sedimentation of particles out of the bags, remineralisation of sedimented moulting products at the bottom of the mesocosm bags was possible during the time period between sampling days. This circumstance, leading to an underestimation of sedimented P bound to moulted carapaces, was, however, certainly more than compensated by the fact that entire (even live) *Daphnia* individuals were removed with the 'sediment water'. Their contribution to sediment P was almost certainly high given that on 28 August the number of *Daphnia* counted on the filters and the amount of sediment P were significantly positively correlated. Yet, despite this fact the amount of sedimented P never exceeded 1% of the total amount of seston P of the overlying water column, leading to the conclusion that, at least for the zooplankton *Daphnia*, sedimentation processes seem a negligible component of the P-cycle.

The strong influence of *Daphnia* on seston stoichiometry may therefore be restricted to the retention of P in *Daphnia* biomass. In this experiment 'retention in biomass' may be primarily defined as the numerical increase of *Daphnia* (population growth). In bags where the numerical increase was highest,

*Daphnia* growth withdrew  $\sim 5$  to  $6 \mu\text{g P L}^{-1}$  from the seston fraction which was evident in both, seston P and TP. Considering  $17 \mu\text{g dry weight ind}^{-1}$  for *D. hyalina* (Santer 1990) and a range of 1.1 to 1.8% P content of dry weight for *Daphnia* (Hessen 1990), complete conversion of  $5.2$  to  $6.3 \mu\text{g P L}^{-1}$  into *Daphnia* biomass would allow for the additional 'growth' of 17 to  $37 \text{ ind L}^{-1}$ . In the highest density treatments (20 and  $40 \text{ ind L}^{-1}$ ) the difference between measured and seeding *Daphnia* densities on 21, 24 and 28 August ranged between 15 and  $44 \text{ ind L}^{-1}$ . Hence, actual *Daphnia* abundances exceeded the calculated 'additional' *Daphnia*  $\text{L}^{-1}$  by only a few  $\text{ind L}^{-1}$ . Clearly, these calculations are highly sensitive to the value of *Daphnia* dry weight they are based on. Yet, they show that the decrease of seston P (and TP) and the numerical increase of *Daphnia* in our experiment may be plausibly explained by the transfer of P from the seston fraction to the zooplankton compartment.

Due to fertilisation, SRP concentrations were high at the beginning of the experiment. Yet, despite increasing P depletion in the seston compartment (indicated by increasing seston C:P ratios), SRP concentrations did not decrease substantially during the course of the experiment, but remained fairly constant. Seston C and, especially, seston P indicate that the particulate fraction was reduced by *Daphnia* grazing to some portion unavailable to *Daphnia*. Besides detritus, this seston fraction probably comprised large dinoflagellates and gelatinous green algae that were not negatively affected by *Daphnia* grazing (Sommer et al. 2001, Chapter III.2). As dinoflagellates have particularly low growth rates (Banse 1982), the compensatory increase of these phytoplankton with *Daphnia* biomass was possibly not enough to substantially withdraw P from the dissolved pool of inorganic P (SRP). Alternatively, phosphate might have been bound to the surface of calcite crystals or co-precipitated together with calcite (Kleiner 1988), especially during the onset of the experiment ('whiting' event). In Lake Constance, up to 50% of the sedimentary flux of P can be related to co-precipitation with calcite (Kleiner and Stabel 1989). Enclosed in the water body of the mesocosm bags, these P-containing particles would be inevitably dissolved at a low pH used in the determination of P (Grasshoff et al. 1999), resulting in unbound orthophosphate. Hence, the relatively high and stable SRP concentrations detected might reflect P bound to calcite particles which were unavailable to phytoplankton throughout the experiment.

In accordance with ecological stoichiometry theory, seston C:P ratios increased linearly with *Daphnia* densities (up to  $40 \text{ ind L}^{-1}$ ), as seston became relatively stronger depleted in P than C in order to meet the high specific P demand of *Daphnia*. As shown above, the transfer of seston P to zooplankton is plausibly explained by *Daphnia* population growth. Highest C:P ratios (up to 306:1) occurred at *Daphnia* densities of  $\sim 30$  to  $50 \text{ ind L}^{-1}$ , which corresponds to threshold C:P ratios of  $\sim 300$  proposed to limit *Daphnia* growth (Olsen et al. 1986, Urabe et al. 1997). At *Daphnia* densities  $> 40$  to  $50 \text{ ind L}^{-1}$ , which represent maximum natural densities of *Daphnia* in Schöensee (Fußmann 1996), C:P ratios were again decreased. The use of such a high zooplankton density resulted not only in a strong decrease of seston P to some unavailable particulate fraction (possibly dinoflagellates and gelatinous green algae), but also in a strong reduction of seston C, resulting in decreased seston C:P ratios. In these treatments,

too, *Daphnia* densities as high as 84 ind L<sup>-1</sup> were observed on 24 August, which again dropped to 67 ind L<sup>-1</sup> on 28 August. Most probably energy constraints (200 to 300 µg C L<sup>-1</sup> of which phytoplankton C represented only 5 to 11%) limited zooplankton growth, especially at these high *Daphnia* densities. In conclusion, the results show that substantial amounts of P may be withdrawn from the seston compartment due to *Daphnia* grazing. P is almost exclusively converted into new zooplankton biomass and allows for zooplankton population growth. At least for *Daphnia*, sedimentation of faecal pellets and moulted carapaces seem negligible processes in pelagic phosphorus dynamics.

### V.3 Hopavågen stoichiometry

In both marine mesocosm experiments, the intention was to test potential stoichiometric effects of two different groups of marine zooplankton on their food: N-rich calanoid copepods and (presumably) P-rich appendicularians (Appendicularian C:P and N:P ratios have, to my knowledge, not been published before). In the Hopavågen study, the appendicularian *O. dioica* developed after ~9 days. While elemental concentrations and stoichiometric ratios could be correlated with the experimentally manipulated densities of the copepod gradient, no *a priori* density gradient could be established for *O. dioica*. Therefore, the natural variation of *O. dioica* densities was used for analysis. Despite reaching peak densities of up to 35 ind L<sup>-1</sup>, no correlations were found for either concentrations of seston C, N and P, or seston stoichiometric ratios with appendicularian densities. Data for the appendicularian treatment are therefore not shown. But possible explanations why for appendicularians did not affect seston stoichiometry are included in the discussion.

Prior to the experiment, I determined the stoichiometry of major zooplankton species and seston in the Hopavågen lagoon. This was done in order to make predictions about the outcome of the experiment. Zooplankton may become limited by the relative elemental content of their food, if the food elemental ratio is higher than the critical food threshold ratio  $Q^*_{F-E}$  (Urabe and Watanabe 1992). If this occurs, the element (N or P) which is more likely to become limiting for phytoplankton production, may be estimated from the stoichiometric imbalance  $\Delta(N:P)_{imb}$ , which is the difference between food and zooplankton N:P ratios (Elser and Hassett 1994). Positive values of  $\Delta(N:P)_{imb}$  indicate P limitation, while negative values point to N limitation.

#### V.3.1 Results

**Stoichiometry in the Hopavågen lagoon** – Body C, N and P content of the most abundant zooplankton, and their relative ratios, are given in Table 10. The C:N ratios of all zooplankton were fairly uniform (C:N~5), and only slightly below the seston C:N (C:N = 7.7). Similarly, zooplankton C:P ratios were lower (C:P<118) than the seston C:P (C:P = 205), with a distinction between copepods (C:P~110) close to the Redfield ratio of C:P = 106, and rapidly reproducing zooplankton - *O. dioica* and *Evadne* sp. – which had C:P ratios (C:P = 87 and 74, respectively) below the Redfield C:P.

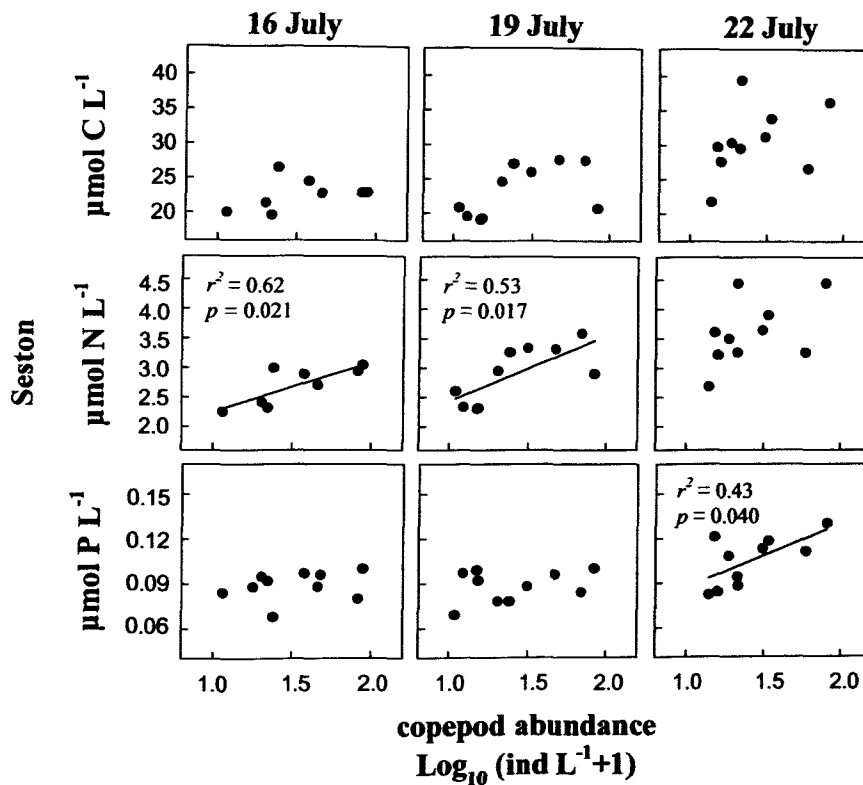
Copepods were, hence, relatively N-rich ( $N:P > 20$ ), whereas *O. dioica* and *Evadne* sp. were balanced ( $N:P \sim 16$ ) with respect to Redfield stoichiometry. For all zooplankton but *Evadne*, critical food threshold ratios with respect to both, N ( $Q^*_{C:N} > 11$ ) and P ( $Q^*_{C:P} > 217$ ), were higher than seston C:N ( $C:N = 7.7$ ) and C:P ( $C:P = 205$ ) ratios, respectively. For *Evadne*, the critical food threshold ratio was slightly lower ( $Q^*_{C:P} = 185$ ) than the seston C:P. This suggests that all zooplankton would primarily face C limitation, and that only *Evadne* (which was not tested in the mesocosms experiments) was likely to become P limited in the Hopavågen. The elemental imbalance  $\Delta(N:P)_{imb}$  was positive for all zooplankton [ $\Delta(N:P)_{imb} = 4$  to 18] due to the lower relative P content in seston ( $N:P = 31$ ) than in zooplankton ( $N:P < 27$ ). Zooplankton were hence expected to preferentially retain P, while releasing relatively more N per unit food in order to maintain elemental balance (Urabe et al. 1995). However, given that C was more likely to become limiting than N or P, effects of zooplankton on seston stoichiometry were not expected.

**Table 10:** Elemental contents and stoichiometry of zooplankton and seston in the Hopavågen on 14 July. C:N ratios were calculated from joint measurements on each filter, whereas C:P and N:P ratios were calculated from all possible combinations of C and P, or N and P, respectively. Values are means  $\pm 1$  SD (in brackets below). Critical food threshold ratios ( $Q^*_{C:N}$ ,  $Q^*_{C:P}$ ) were calculated according to Urabe and Watanabe (1992) assuming estimated minimal ( $\sim 20\%$ ) and maximal ( $\sim 40\%$ ) values of C gross growth efficiency (Straile 1997). The  $\Delta(N:P)_{imb}$  is the difference of mean seston N:P and mean zooplankton N:P ratio. Zooplankton  $> 150 \mu m$  was dominated by copepods, but also included *Evadne*. Symbols are: n (number of replicate samples), Ind (number of individuals per sample), - (calculation not possible), n.d. (not determined).

taxon/fraction	n	Ind	$\mu g C ind^{-1}$	$\mu g N ind^{-1}$	$\mu g P ind^{-1}$	C:N	C:P	N:P	$Q^*_{CN}$	$Q^*_{CP}$	$\Delta(N:P)_{imb}$
<i>Temora longicornis</i>	4	34-38	7.1 (0.6)	1.8 (0.2)	0.17 (0.03)	4.8 (0.1)	110 (18)	27 (5)	12-24	275-550	4.2
<i>Centropages hamatus</i>	4	39-41	4.7 (0.4)	1.2 (0.1)	0.12 (0.01)	4.5 (0.1)	105 (9)	23 (2)	11-23	263-525	7.8
zooplankton $> 150 \mu m$	4	n. d.	-	-	-	5.2 (0.1)	118 (22)	26 (5)	-	-	4.7
<i>Oikopleura dioica</i>	3	31-34	3.2 (0.6)	0.8 (0.2)	0.11 (0.01)	4.9 (0.2)	87 (12)	18 (3)	12-25	217-435	13.2
<i>Evadne</i> sp.	3	70-92	2.1 (0.8)	0.4 (0.1)	0.07 (0.01)	5.7 (0.4)	74 (30)	13 (5)	14-29	185-370	18.0
Seston $< 100 \mu m$	4	-	-	-	-	7.7 (0.4)	205 (25)	31 (3)	-	-	-

**Seston** - In the copepod treatment, start concentrations of C, N and P in seston were low, ranging from 20 to 26  $\mu mol C L^{-1}$ , 2.2 to 3.0  $\mu mol N L^{-1}$ , and 0.09 to 0.13  $\mu mol P L^{-1}$  (Figure 20). Start seston N on

16 July was significantly positively correlated with increasing copepod abundance. Along the course of the experiment, seston C, N and P concentrations remained more or less constant at low copepod densities, but increased sometimes strongly at medium to high densities. This increase in seston C, N and P with time was, however, not strictly a function of copepod densities, because seston C, N and P were only rarely correlated to copepod abundance. Significant correlations were, however, found for seston N and P content on 19 and 22 July, respectively, explaining ~50% of seston N and P variability.

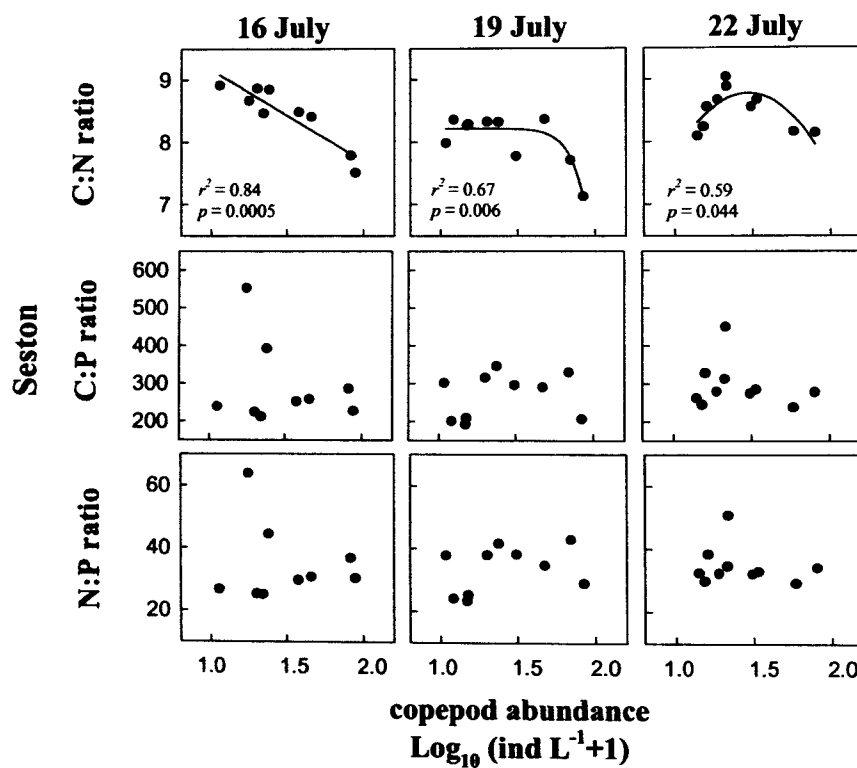


**Figure 20:** Seston C (top), seston N (middle) and seston P (bottom) against  $\text{Log}_{10}$ -converted copepod densities. Linear regressions for seston N and seston P are:  $y=1.5+0.8x$  (16 July),  $y=1.3+1.1x$  (19 July) and  $y=0.04+0.04x$  (22 July), respectively.

Initial seston C:N ratios ranged from 7.5 to 8.9 and showed a highly significant, negative correlation with copepod abundance (Figure 21). This was most probably an undesired 'enrichment effect' produced by the addition of water (together with copepods) from the barrel in which copepods had been concentrated prior to their addition (Chapter II.2). Ammonia released from zooplankton within the barrels probably enhanced nutrient concentrations proportionately as copepods were added. This additional N thus probably produced increasingly N-enriched seston as it was incorporated rapidly into phytoplankton. Note, however, that this 'enrichment' only affected seston C:N, but not C:P ratios.



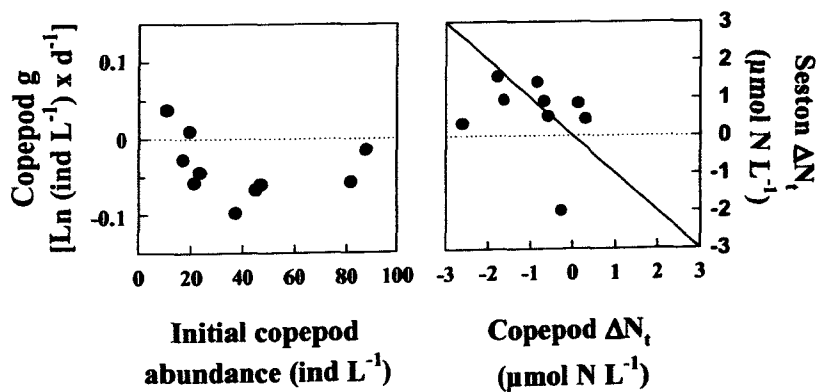
Correspondingly, seston N, but not P concentrations were also significantly enriched with copepod abundance on 16 July (Figure 20). On 19 July, seston C:N ratios decreased to a uniform value of  $\sim 8.3$  at copepod densities  $<25$  ind  $L^{-1}$ , and showed a positive, almost linear relationship with copepod densities  $<25$  ind  $L^{-1}$  on 22 July (Figure 21). At highest copepod densities ( $>50$  ind  $L^{-1}$ ) seston C:N ratios remained low on 19 July (C:N = 7.1 to 7.7), but increased slightly (C:N $\sim 8.1$ ) on 22 July. The functions shown in Figure 21 were fitted to all data. Seston C:P and N:P ratios showed no correlation with copepod densities on any day. They generally ranged from  $\sim 200$  to 350 and from  $\sim 25$  to 40, respectively, and - as in the Hopavågen lagoon - indicated relative P deficiency in seston with respect to both, C (C:P> Redfield of 106) and N (N:P> Redfield of 16).



**Figure 21:** Seston C:P (top), seston C:N (middle) and seston N:P ratios (bottom) against  $\text{Log}_{10}$ -converted copepod densities. Regressions for seston C:N ratio are:  $y=10.6-1.5x$  (linear regression, 16 July),  $y=8.2-0.0001x^{19}$  (power function, 19 July), and  $y=-0.6+13.7x-4.3x^2$  (polynomial second order function, 22 July).

**Dissolved inorganic nutrients** - Concentrations of dissolved nutrients in the bags were generally low ( $<0.3 \mu\text{mol NH}_4 L^{-1}$ ) or practically undetectable ( $<0.10 \mu\text{mol NO}_3$ ,  $<0.1 \mu\text{mol PO}_4 L^{-1}$ ), and showed no correlations with copepod densities (Figure not shown).

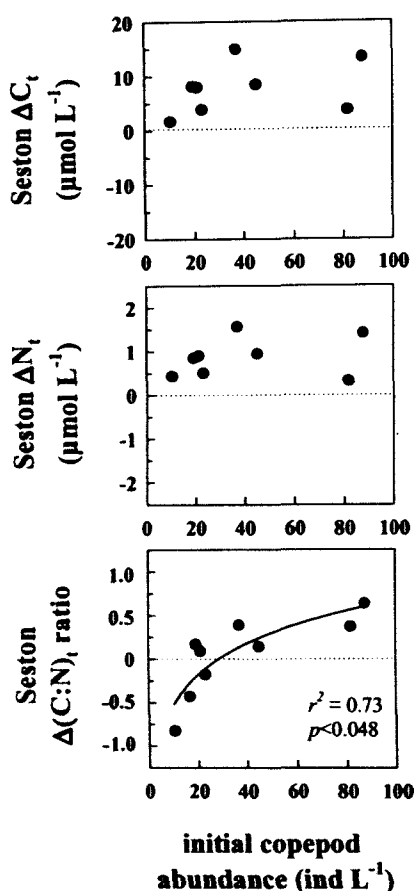
**Copepod growth rate** – The copepod populations generally suffered from mortality (Figure 22, left). Except for two bags at low copepod densities, absolute copepod growth rates were all negative. Due to the absence of diazotrophic algae (Chapter IV.4) and the practically undetectable amounts of dissolved inorganic nutrients, decreases in copepod abundance ( $\Delta\text{Cop}_t$ ) may be expected to be paralleled by increases in seston N ( $\Delta\text{N}_t$ ). In Figure 22 (right), seston  $\Delta\text{N}_t$  is shown as a function of  $\Delta\text{Cop}_t$ , after converting  $\Delta\text{Cop}_t$  to units of N (assuming  $1.5 \mu\text{g}$  or  $0.11 \mu\text{mol N copepod}^{-1}$ , Table 10). In some bags, decreases of  $\Delta\text{Cop}_t$  in terms of N, were paralleled by increases of seston  $\Delta\text{Cop}_t$ , as indicated by the function  $y=-x$ . Yet, especially in bags of the highest copepod densities, where copepod mortality was relatively low (Figure 22, left), increases of seston  $\Delta\text{N}_t$  were proportionately much stronger (see below). In turn, the highest copepod mortality at intermediate copepod densities (Figure 22, left), yielded an estimated maximum release of  $2.6 \mu\text{mol N L}^{-1}$  from copepods. This was lower than the maximum increase of seston  $\Delta\text{N}_t$  ( $1.6 \mu\text{mol L}^{-1}$ ). Given that copepod N bound in the carapace may possibly not be completely and immediately recycled, and that detritus accumulated visibly at the bottom of the enclosure bags, the release of copepod N may not be completely mirrored in increased seston  $\Delta\text{N}_t$ . Similar calculations for seston P, showed a large scatter of data (not shown).



**Figure 22:** The absolute copepod growth rate  $g$  as a function of initial copepod abundance on 16 July (left), and the difference between final and initial seston N (seston  $\Delta\text{N}_t$ ) as a function of changes in copepod abundance ( $\Delta\text{Cop}_t$ ) (right). The line represents the function  $y=-x$ , which would indicate the complete transfer of N between copepods and seston. All data were calculated from 16 to 22 July. The dotted horizontal line marks 0.

**Relative changes in seston** – The difference between initial and final seston C ( $\Delta\text{C}_t$ ), seston N ( $\Delta\text{C}_t$ ) and seston C:N [ $\Delta(\text{C:N})_t$ ] ratios are shown in Figure 23. Both, seston  $\Delta\text{C}_t$  and seston  $\Delta\text{N}_t$  were increased in all bags. At copepod densities  $<50 \text{ ind L}^{-1}$ , seston  $\Delta\text{C}_t$  and seston  $\Delta\text{N}_t$  increased

approximately proportionately with increasing copepod abundance. In contrast to seston  $\Delta C_t$  and  $\Delta N_t$ , seston  $\Delta(C:N)_t$  ratios increased significantly with copepod densities along the entire copepod gradient. This increase was due to relatively weaker increases of seston  $\Delta C_t$  than seston  $\Delta N_t$  at low copepod densities [negative  $\Delta(C:N)_t$ ], and relatively stronger increases of seston  $\Delta C_t$  than seston  $\Delta N_t$  at high copepod densities [positive  $\Delta(C:N)_t$ ], respectively. Using mean or final copepod densities instead of initial densities as abscissa values, yielded similar, significantly positive correlations.



**Figure 23:** Seston  $\Delta C_t$  (top), seston  $\Delta N_t$  (middle), and seston  $\Delta(C:N)_t$  (bottom) as a function of the initial copepod abundance on 16 July. All data were calculated from 16 to 22 July. The power regression is:  $y = -12.0 + 10.4x^{0.04}$ . The dotted horizontal line marks 0.

### V.3.2 Discussion

**The copepod treatments** –Copepod mortality was an important characteristic of the experiment. It was probably the result of energy constraints for copepods. The calculated C content of dietary particles (diatoms and ciliates; nanoflagellates may be considered too small for copepod ingestion, Berggreen et al. 1988) was  $\sim 3$  to  $5 \mu\text{g C L}^{-1}$  on 16 July (Chapter III.3). Assuming a daily C ingestion of 7 to 18% body C content (Kiørboe et al. 1985) and 4.7 to  $7.1 \mu\text{g C content copepod}^{-1}$  (Table 10), copepod densities of  $> \sim 4$  to  $15 \text{ ind L}^{-1}$  would remove 100% of the dietary C standing stock within only a day. Clearly, phytoplankton productivity, the use of storage lipids, and starvation tolerances of typically  $\sim 3$  to 10 d (Mauchline 1998) - which encompasses the duration of the experiment (6 d) -

probably 'reduced' potential copepod mortality. These calculations, however, fit relatively well with the densities of copepods in the two bags where absolute copepod growth rates were positive. Here, initial densities were very low (10 and 19 ind L<sup>-1</sup>, respectively) and increased only marginally (13 and 20 ind L<sup>-1</sup> on 22 July, respectively). In all other bags, copepod populations suffered from sometimes high mortality, with final densities being reduced by 8 to 44% of the initial abundance.

Copepod mortality may be a negligible factor if zooplankton represents only a small fraction of the total N and P pool (here, including zooplankton). However, copepods represented – depending on treatment density – 11 to 51% of the total N (assuming 1.5 µg N ind<sup>-1</sup>) and 13 to 55% of the total P pool (assuming 0.15 µg P ind<sup>-1</sup>). Therefore, the maximum release of copepod N and P to seston due to mortality may be estimated of being ~2.6 µmol N L<sup>-1</sup> and ~0.11 µmol P L<sup>-1</sup>. This corresponds to ~14% and ~15% of the total N and P pools, respectively.

The main transfer of elements between seston and copepods was thus apparently not directed from seston to copepods (element retention in copepod biomass), but from copepods to seston (element release due to copepod mortality). This was indicated by increases of seston N (and also P) concentrations, especially at high copepod densities (Figure 20). However, changes in seston stoichiometry were not found for P, but exclusively for N. This indicates that phytoplankton growth was limited by N. The significant negative linear correlation of seston C:N ratios with copepod abundance after the addition of copepods clearly demonstrated this. Phytoplankton N-limitation was also indicated by phytoplankton growth responses in short-term bioassays (Sommer et al., submitted), and by the response of phytoplankton to fertilisation of the mesocosms after termination of the experiment (data not shown). Phytoplankton N-limitation was unexpected since both, seston C:P (C:P > ~200) and seston N:P ratios (N:P > ~25) were higher than the Redfield ratio, indicating that P was more likely to limit phytoplankton growth. One may speculate that the 'true' values of seston N:P ratios may have been distorted by a high contribution of some particulate N fraction unavailable to phytoplankton, so that seston only 'seemed' relatively depleted in P with respect to N. The absence of macroscopically visible detritus in the microscope (e.g. copepod moulting products) gave no support to this notion. Alternatively, N requirements of phytoplankton (e.g. nanoflagellates) in the Hopavågen may have been especially high (N:P > 16). Smith (1982) showed that phytoplankton species may differ in their optimum N:P demand, which can be markedly above the Redfield ratios (N:P > 20). Whatever the reason, three lines of evidence showed that phytoplankton growth was indeed limited by N and not P, as expected from stoichiometric ratios of seston.

Given that the enclosure bags represented a closed system for N cycling (absence of diazotrophic algae, bags sealed at the bottom), copepod mortality ( $\Delta\text{Cop}_i$ ) should be mirrored in increases of seston N ( $\Delta\text{N}_i$ ). This was indeed apparent in some, but not all bags (Figure 23). Incomplete recycling of dead copepods, the accumulation of detritus at the bottom of the enclosure bags and somatic growth of copepods (not quantified), may be possible reasons why N mass balance calculations did not fit in all cases. Nevertheless, it seems reasonable to assume that the release and subsequent transfer of N from

dead copepods to seston caused the increases of seston N, especially, as diazotrophic algae seemed to be absent (Chapter IV.4).

Seston  $\Delta C_t$  equally increased along the copepod density gradient. Assuming, for the moment, that differences in seston  $\Delta C_t$  were equally caused by the transfer of (dead) copepod C to seston, seston  $\Delta(C:N)_t$  would be expected to show no correlation with copepod abundance. This is because copepod C and N would be transferred to seston in a fixed ratio, namely the copepod body C:N (C:N~5, Table 10). Yet, in contrast to N, C cannot be considered to cycle within a closed system, because C may leave (e.g. respiration) and enter the pelagic system (e.g. photosynthesis). Since phytoplankton was demonstrated to be limited by N, the release of copepod N was likely to cause increases in seston N due to increased C fixation by phytoplankton. Yet apparently, the relative increases of seston C and N were not proportional, but dependent on copepod density: As copepod abundance increased, the increase of C was proportionally higher with respect to the increase of N in seston. Copepods thus caused a relative depletion of N in seston, and enhanced, not reduced, N limitation of phytoplankton.

This finding is seemingly consistent with the theory of ecological stoichiometry, which states that zooplankton may enhance, not decrease, the nutritional status of phytoplankton (Sterner 1990, Elser and Urabe 1999). However, the biological mechanism proposed by ecological stoichiometry is that zooplankton preferentially retain the nutrient in demand and recycle the nutrient in excess (Urabe et al. 1995), if the food elemental ratio is higher than the critical food threshold ratio (Urabe and Watanabe 1992). In the case of copepods, even the most conservative estimates of the N and P food threshold ratios were higher than the seston C:N or C:P ratios, respectively (Table 10). If we, moreover, consider that not all C in seston was edible by copepods (e.g. nanoflagellates, possibly some detritus fraction) and that copepod mortality was likely to enrich seston with substantial amounts of N and P, we may assume that 'true' seston C:N and C:P ratios (those experienced by copepods throughout the experiment) were even lower than start seston ratios. This suggests that copepods were strongly limited by C, which was reflected in, generally, high copepod mortality. Indeed, copepod mortality would even be expected to reduce, not enhance N limitation of phytoplankton, because copepods were relatively richer in P than seston (Table 10). The increase of seston  $\Delta(C:N)_t$  ratios with copepod density was therefore not the result of preferential nutrient recycling by copepods.

Rather, it seems that the biological mechanism causing increased N-limitation with copepod density was that copepods caused substantial changes in food web structure. Copepods reduced ciliate biomass and, therefore, presumably released nanoflagellates from ciliate grazing pressure via a trophic cascade (Chapter III.3). The increase of C due to nanoflagellate growth was higher, the more their growth was fuelled by N from dead copepods (Figure 23, top and middle), yet always proportionately higher, the more copepods were present (Figure 23, bottom). This suggests that the physical elimination of ciliates alone - even if new nutrients are added (from dead copepods) - may sufficiently explain the observed increase of phytoplankton N-limitation: (Nano)phytoplankton growth continued at the expense of the

intracellular nutrient pool, until the minimal cell quota was reached. This ultimately led to increased seston  $\Delta(\text{C:N})_t$  ratios.

These findings may imply that phytoplankton growth in marine oligotrophic systems may become limited by N, even if N is maintained within the euphotic zone. In the subtropical oligotrophic ocean, phytoplankton is generally believed to become limited by N due to the export of organic matter (algae, dead copepods, exuviae and, especially, copepod faecal pellets) and - with it - of N to below the euphotic zone. The experimental setup used here did not allow for this export of N, so that seston became 'fertilised' with N (from dead copepods) in the mesocosms. Yet, increases of seston with N was more than compensated by increased C concentrations. Clearly, this does not explain why phytoplankton growth in marine oligotrophic regions becomes limited by N in the first case. It, however, demonstrates that existing N-limitation of phytoplankton may become enhanced despite the retention of N in the upper water layer, simply because copepods cause a shift in phytoplankton size structure.

**The appendicularian treatments** - Stoichiometric effects, if any, were predicted for seston P, not N stoichiometry. Therefore, it is not surprising that appendicularians, with even less pronounced N requirements than copepods (due to the larger stoichiometric imbalance), showed no effect on seston N stoichiometry. Yet, may we generally even expect a stoichiometric impact of appendicularians on seston?

Appendicularians have high population growth rates (Paffenhöfer 1973), resulting in a relatively high body P content (Main et al. 1997) and, consequently, low C:N:P ratios (Table 10). This makes them stoichiometrically similar to freshwater cladocerans, for which substantial transfer of P, and resulting increases of seston C:P were shown (Chapter V.2.2). However, *Daphnia* differ in that - due to a high biomass (estimated as  $\sim 17 \mu\text{g}$  dry weight  $\text{ind}^{-1}$ ) - P content (estimated as  $\sim 2 \mu\text{g}$  P  $\text{ind}^{-1}$ ; 1.1 to 1.8% P content of dry weight: Hessen 1990) is by a factor of  $\sim 20$  higher than that of the (adult) appendicularian *O. dioica* ( $0.11 \mu\text{g}$  P  $\text{ind}^{-1}$ , Table 10). After removing copepods in the appendicularian treatment, juvenile *O. dioica* developed in the bags, being of approximately half the size of adult *O. dioica* measured earlier in the Hopavågen. Assuming 25% body P content of adult *O. dioica*, the development of peak *O. dioica* densities ( $35 \text{ ind L}^{-1}$ ) in the bags would result in a maximum P drain from seston of only  $\sim 1 \mu\text{g}$  P  $\text{L}^{-1}$  (or  $\sim 0.03 \mu\text{mol}$  P  $\text{L}^{-1}$ ). This is low with respect to the P drain caused by *Daphnia* at a numerically comparable population increase ( $\sim 6 \mu\text{g}$  P  $\text{L}^{-1}$ , Chapter V.2). The estimated P drain due to *O. dioica* population increase, thus, represents  $\sim 20$  to 25% of initial total P in the appendicularian treatment. In comparison, copepod P in the copepod treatment was estimated to represent 11 to 51% of the total P pool, and P release due to mortality, was estimated to be as high as  $0.4 \mu\text{mol}$  P  $\text{L}^{-1}$ , more than 13 times the amount required by the maximum development of *O. dioica* ( $\sim 0.03 \mu\text{mol}$  P  $\text{L}^{-1}$ ). As shown above, even copepods had no effect on P stoichiometry.

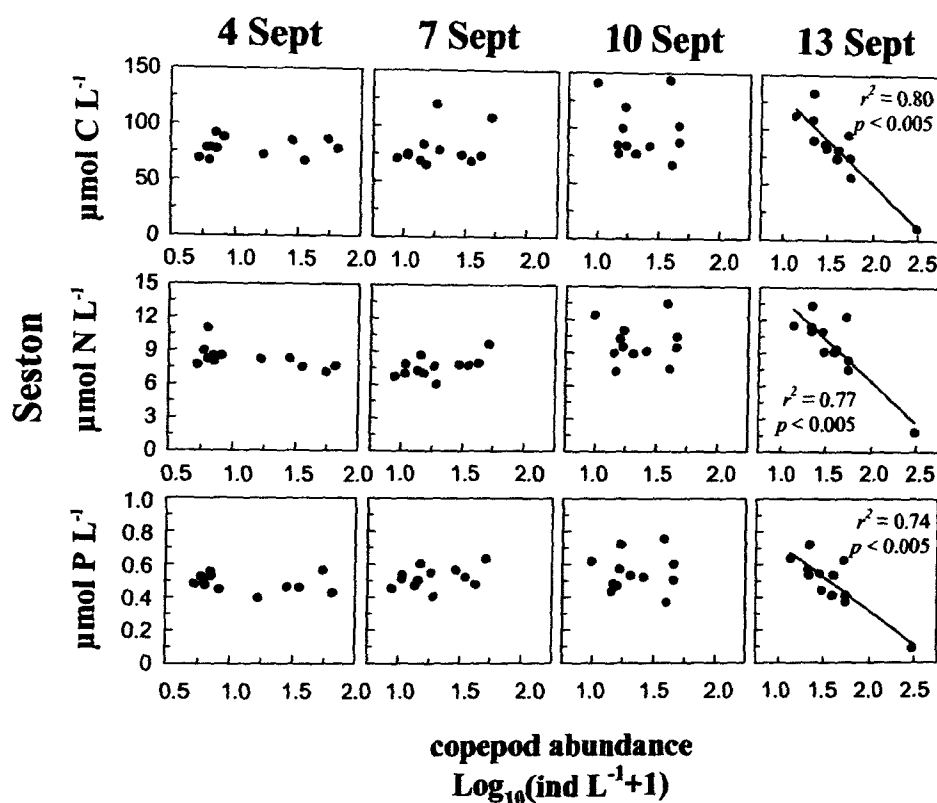
In the ocean, maximum densities of  $\sim 100 \text{ ind L}^{-1}$  have been reported for *O. dioica* (Nakamura 1998), which, even so, correspond to P requirements of only  $\sim 11 \mu\text{g}$  or  $\sim 0.34 \mu\text{mol P L}^{-1}$  (assuming  $0.11 \mu\text{g P content ind}^{-1}$ ). In the Baltic (Behrends 1997), or elsewhere (Laundry 1994), *O. dioica* is usually very much less frequent ( $< 30 \text{ ind L}^{-1}$ ). Therefore, it may be concluded that the development of *O. dioica* populations in the sea may not represent a substantial P drain for phytoplankton. Despite their similar stoichiometry, appendicularians are apparently not stoichiometric equivalents of freshwater cladocerans in the marine environment.

#### V.4 Kiel Fjord stoichiometry

This marine mesocosm experiment differed strongly from the Hopavågen study, in that the phytoplankton community was more diverse (Chapter II.4), and nutrient concentrations were elevated. Due to this circumstance and the higher water temperature ( $\sim 20^\circ\text{C}$ ), the Kiel Fjord experiment took place under similar physical conditions (apart from salinity) as the Schöhsee experiment (Table 1). Most of all, it was characterised by the development of the  $\text{N}_2$ -fixing (diazotrophic) and potentially toxic cyanobacterium *Nodularia spumigena* (Chapter II.4). This phytoplankton markedly affected nitrogen stable isotope signatures (Chapter IV.5), indicating that ‘new’ N was introduced to the pelagic system.

##### V.4.1 Results

**Seston** - Start concentrations of seston C, N and P in the Kiel Fjord experiment ranged from 67 to  $90 \mu\text{mol C L}^{-1}$ , 7 to  $11 \mu\text{mol N L}^{-1}$ , and 0.40 to  $0.56 \mu\text{mol P L}^{-1}$  (Figure 24). Thus, C, N and P content in seston was approximately three to four-fold (C and N), or four to five-fold (P) of seston concentrations in the Hopavågen (Figure 20). On 10 September, the scatter of data for seston C, N and P concentrations, became higher, yet showed no correlation with copepod abundance. On 13 September, seston C, N and P concentrations were significantly negatively correlated with copepod abundance, resulting from both, increases at low copepod densities and decreases at high densities. The increases at low copepod densities were especially pronounced for seston C ( $\sim 30 \mu\text{mol L}^{-1}$ ) and seston N ( $\sim 4 \mu\text{mol L}^{-1}$ ) with respect to start concentrations, but were only marginal for seston P ( $\sim 0.1 \mu\text{mol L}^{-1}$ ). Note, however, that ‘low’ copepod densities were not identical for all days (apparent in the scaling of the abscissa), as copepod populations increased over time (see also below). The strongest depletion of seston C, N and P occurred in bag 24 (right-most data in Figure 24), where copepod growth was highest (see below). If data from this bag were excluded, linear correlations were still statistically significant ( $p < 0.05$ ), yet explained less of data variation ( $r^2 = 0.37$  to  $0.55$ ).

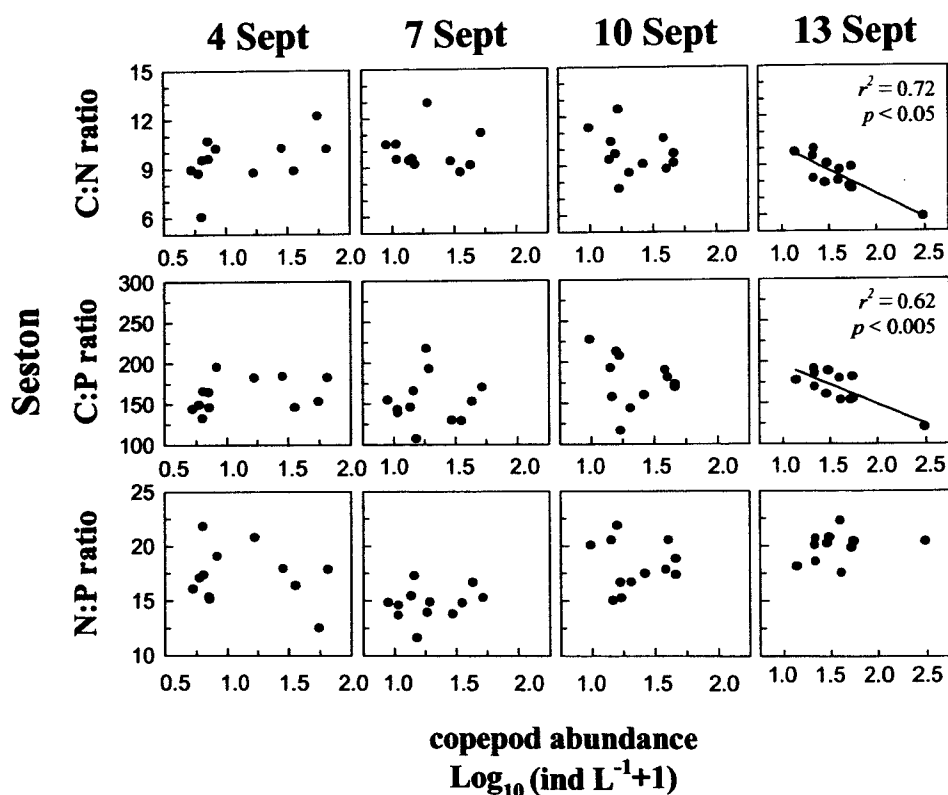


**Figure 24:** Seston C (top), seston N (middle) and seston P (bottom) against  $\text{Log}_{10}$ -converted copepod densities. Linear regressions for seston C, N and P on 13 September are:  $y=211-81x$ ,  $y=22-8x$  and  $y=1.1-0.4x$ , respectively. Note the different scaling of the abscissa for different days.

Initial seston C:N, C:P and N:P ratios scattered widely (C:N= 6 to 12, C:P= ~130 to 200, and N:P=13 to 22, respectively) (Figure 25). They indicated relative deficiencies of both, N (C:N = generally >8) and P (C:P = generally >140) with respect to C in seston (assuming optimal Redfield stoichiometry of C:N = 6.6 and C:P = 106). Seston N:P ratios was balanced to only marginally elevated (N:P= ~15 to 20) with respect to the Redfield N:P ratio. On the following two sampling days (7 and 10 September), the scatter of seston C:N and C:P ratios remained similar in magnitude and range. In contrast, seston N:P ratios first decreased on 7 September (N:P = generally <16), and thereafter again increased on 10 September (N:P = generally >16) and 13 September (N:P = generally >18), respectively. The initial decrease of seston N:P was accompanied by decreases in copepod abundance (copepod mortality), especially in bags of high copepod densities. Thereafter, copepod population growth was positive (apparent in the scaling of the abscissa), and seston N:P ratios increased. On 13 September, seston C:N and C:P ratios were significantly positively correlated with copepod densities. The decrease of seston C:N and C:P ratios with copepod abundance was, hence, due to relatively stronger decreases of seston

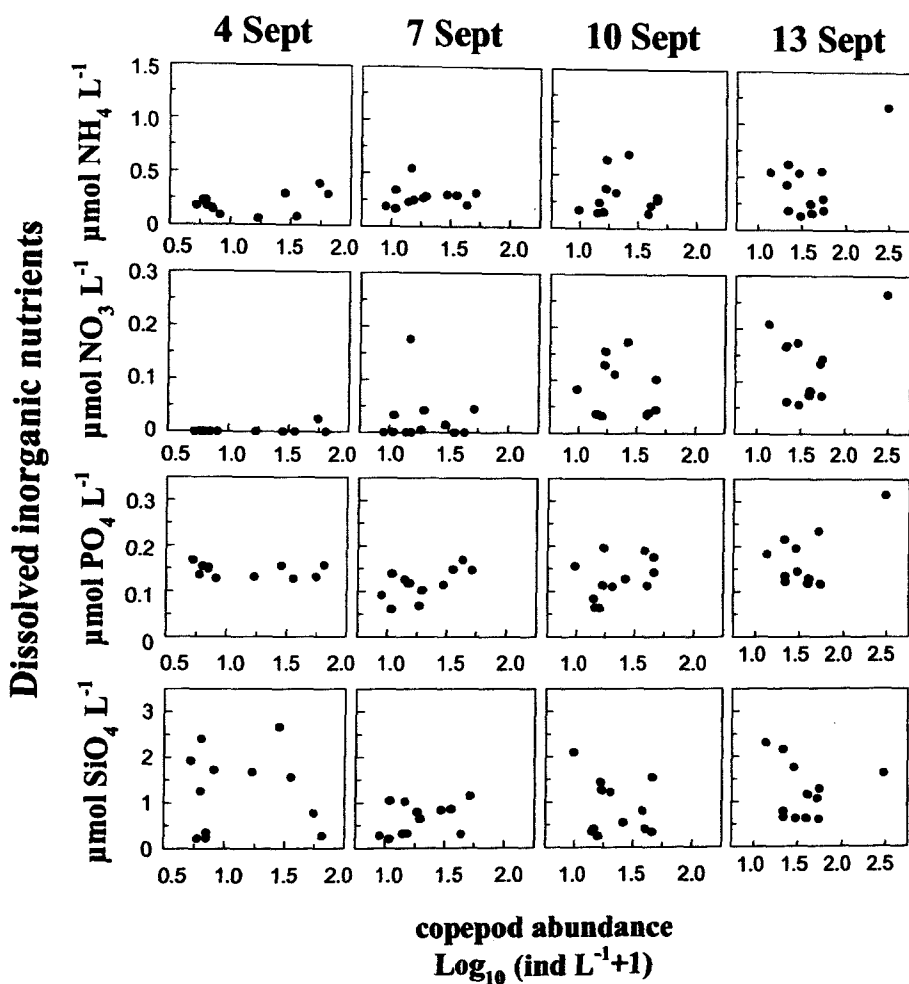


C than seston N or seston P, respectively (Figure 24). No correlation of seston N:P ratios with copepod abundance was evident.



**Figure 25:** Seston C:P (top), seston C:N (middle) and seston N:P ratios (bottom) against  $\text{Log}_{10}$ -converted copepod densities. Linear regressions for seston C:N and C:P ratios on 13 September are:  $y = 12.9 - 2.8x$  and  $y = 242 - 47x$ , respectively. Note the different scaling of the abscissa for different days.

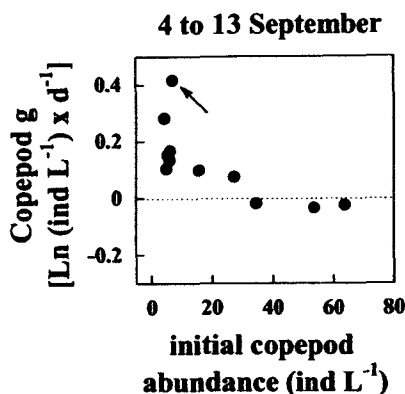
**Dissolved inorganic nutrients** - Start concentrations of ammonia and nitrate were low ( $< 0.5 \mu\text{mol NH}_4 \text{ L}^{-1}$ ) or practically undetectable (Figure 26). Yet, initial phosphate ( $0.13$  to  $0.17 \mu\text{mol PO}_4 \text{ L}^{-1}$ ) and also silicate ( $0.3$  to  $2.7 \mu\text{mol SiO}_4 \text{ L}^{-1}$ ) concentrations were high. Along the course of the experiment, all nutrient concentrations increased - most markedly nitrate - and showed no correlation with copepod densities. Concentrations of ammonia, nitrate and phosphate were exceptionally high in bag 24 on 13 September (right-most data points in Figure 26).



**Figure 26:** Concentrations of dissolved inorganic nutrients against  $\text{Log}_{10}$ -converted copepod densities. Note the different scaling of the abscissa for different days.

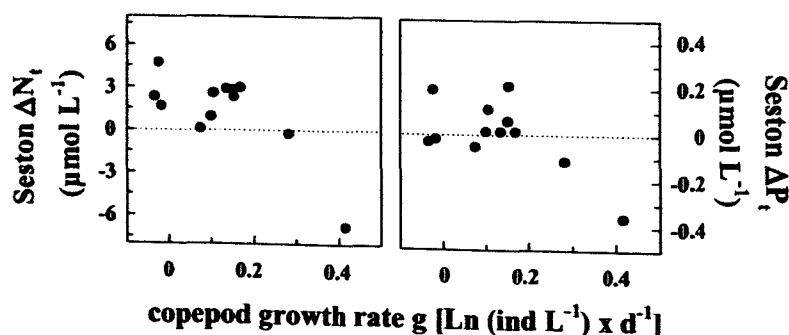
Ratios of dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_3 + \text{NH}_4$ ) to dissolved inorganic phosphorous (DIP) were low on 4 September (range: 0.7 to 3.2) and relatively higher on 13 September (range: 1.3 to 5.9, Figure not shown). This was due to relatively stronger increases of DIN than DIP with time (apparent in Figure 26). Yet, DIN: DIP ratios were at all times lower than the Redfield N:P of 6.6, indicating relative N deficiency.

**Copepod growth rate** – Absolute copepod growth rates were positive for copepod densities  $<30$  ind  $L^{-1}$  and slightly negative at copepod densities  $>30$  ind  $L^{-1}$  (Figure 27). Highest growth occurred in bag 24 (0.42  $d^{-1}$ ), where copepods increased from initially 7 ind  $L^{-1}$  to 303 ind  $L^{-1}$  on 13 September.



**Figure 27:** The absolute copepod growth rate  $g$  calculated from 4 to 13 September as a function of initial copepod densities. The dotted horizontal line marks zero copepod growth. The arrow points to bag 24.

The difference between start and final concentrations of seston N (seston  $\Delta N_t$ ) and of seston P (seston  $\Delta P_t$ ) showed that both, seston N and P content increased over time in most bags (Figure 28). If data from bag 24 were excluded, correlations of both, seston  $\Delta N_t$  and  $\Delta P_t$  with absolute copepod growth rates were not significant ( $r^2 < 0.30$ ,  $p > 1.0$ , not shown). This indicates that (moderate) copepod reproductive growth and, hence, the retention of elements (N and P) in copepod tissue was not a stoichiometrically important process. Only exuberant copepod reproductive growth, as it occurred in bags 24 (where copepods significantly reduced all particles, including *N. spumigena*, Chapter III.4), proved to substantially reduce seston N and P content (right-most data points in Figure 28). While nitrogen fixation by *N. spumigena* probably accounted for the increases in seston N with time, (although a linear correlation of *N. spumigena* cell concentrations on 13 September with seston  $\Delta N_t$  showed no correlation), increases of seston  $\Delta P_t$  may have resulted from phytoplankton P uptake from the dissolved inorganic pool.



**Figure 27:** Seston  $\Delta N_t$  (left), and seston  $\Delta P_t$  (right) as a function of absolute copepod growth rates  $g$ . All data were calculated from 16 to 22 July. The dotted horizontal line marks 0.

#### V.4.2 Discussion

At the start of the mesocosm experiment, seston in Kiel Fjord was quantitatively rich, yet relatively deficient in both, N and P with respect to C. This was evident in that seston C:N (C:N~ 8 to 12) and C:P ratios (C:P~ 140 to 200) were higher than the Redfield ratio (C:N = 6.6, C:P = 106). Seston N:P ratios (N:P = 13 to 22), however, suggest that N and P in seston were present at a balanced ratio (N:P=16). Since quantities of detritus may be assumed to be high in Kiel Bight, its contribution to seston C may have 'distorted' seston C:N and C:P ratios by increasing them over the Redfield ratio. Thus, we may assume that phytoplankton growth at the onset of the experiment was not limited by nutrients. The converse assumption - a low contribution of detritus - would suggest that phytoplankton growth was limited by N, rather than by P. This stems from the observation that the ratios of DIN:DIP on 4 September were low (0.7 to 3.2), because of high phosphate concentrations ( $>0.1 \mu\text{mol L}^{-1}$ ). Consequently, phosphate had been incorporated by phytoplankton until balanced N:P ratios were achieved, while excess phosphate accumulated as DIP. However, measurable amounts of ammonia at the start of the experiment (up to  $0.5 \mu\text{mol NH}_4 \text{L}^{-1}$ ), and increasing concentrations of both, ammonia and nitrate during the experiment suggest that N was not limiting phytoplankton growth.

Predictions of which element is likely to become limiting for phytoplankton growth is based on the difference between resource and zooplankton N:P ratios, termed the stoichiometric imbalance  $\Delta(\text{N:P})_{\text{imb}}$  (Elser and Hassett 1994). Zooplankton in the Kiel Fjord experiment consisted mainly of the calanoid copepod *A. clausi*. Since body N:P ratios of *Acartia* species generally range from ~14 to 22 (Gismervik 1997, Walve and Larsson 1999, Petrola et al. 2002) and seston N:P ratios here were within the same range (N:P = 13 to 22) as zooplankton N:P ratios, the stoichiometric imbalance in the enclosure bags was probably negligible. Hence, copepods would supply phytoplankton with released nutrients in the same ratio as required by phytoplankton. Yet, even if we assume that the body N:P ratio of copepods was slightly higher than seston N:P ratios, phytoplankton would not have become limited by N due to the retention of N in copepod tissue. This is because 'new', atmospherically derived N was likely to enter the planktonic system via N fixation by *N. spumigena*. Nitrogen stable isotope measurements (Chapter IV.5), the general increase of seston N with time (seston  $\Delta\text{N}_t$ ), and of DIN concentrations indicate that this indeed happened. Possibly, diazotrophic N fixation even exceeded N retention in copepods, since seston  $\Delta\text{N}_t$  was not correlated with absolute copepod growth rates (except for bag 24), but increased independently of it in nearly all bags. The large DIP pool ( $\sim 0.05$  to  $0.2 \mu\text{mol P L}^{-1}$ ) would furthermore supply phytoplankton with P proportionately as N is fixed, resulting in a seston N:P stoichiometry close to the Redfield ratio.

Seston C:N and C:P ratios were, however, affected by copepods. Negative correlations of seston C:N and C:P ratios with copepod densities on 13 September resulted from relatively stronger decreases of seston N or P with respect to seston C, respectively. In turn, decreases of seston N and P were balanced, as seston N:P ratios showed no correlation with copepod densities. These results are seemingly consistent with the classical view of zooplankton grazing which considers grazing a

mechanism of reducing nutrient stress as zooplankton fertilise phytoplankton with nutrients (Sterner 1986), or prevent phytoplankton from 'diluting' their cell-quotas by biomass (C) increase not balanced by concomitant nutrient uptake (Sterner 1989). Consequently, copepods in the Kiel Fjord experiment would be seen to reduce nutrient limitation (N and P) of phytoplankton. However, as outlined above, phytoplankton growth was probably not at all nutrient limited in the enclosure bags, despite seemingly high seston C:P and C:N ratios. Thus, the conclusion cannot be drawn that phytoplankton nutrient limitation was relieved by copepod grazing, although copepods decreased seston C:N and C:P ratios. Instead, this may be explained by the reduction of detritus within the seston fraction due to, possibly, enhanced bacterial degradation or direct filtration of detritus (Buskey et al. 1999). This notion is also consistent with my personal observation that plankton samples of high copepod treatments seemed 'clearer' in the counting chambers.

Therefore, it may be concluded that copepods had no negative effect on the nutritional (elemental) status of phytoplankton in the Kiel Fjord experiment. This was probably the result of a negligible stoichiometric imbalance between phytoplankton and zooplankton (similar N:P ratios). Additionally, the presence of both, a N source (*N. spumigena*) and a P pool (DIP) which could easily 'compensate' for marginal elemental deficiencies in the nutrient supply ratio of phytoplankton.

## V. 5 Summary and conclusions

The fundamental mechanism proposed to cause consumer-driven nutrient limitation in phytoplankton is that elements in excess of consumer demands are released, whereas the element in highest demand is retained in consumer biomass (Elser and Urabe 1999). This concept was tested in the mesocosm experiments for zooplankton differing in their N:P ratios (copepods, cladocerans, appendicularians) under different degrees of trophic and food web composition. The results of all mesocosm experiments will be discussed here under four essential aspects of this concept:

(1) *There must be a difference between the food and grazer N:P ratios, the so called stoichiometric imbalance (Elser and Hassett 1994).*

In the Schöhsee experiment, seston N:P ratios were ~18 to 24 on 21 July (data not shown, the P deficiency was due to the removal of sediment in the enclosures, Chapter II.2), while *Daphnia* N:P ratios may be considered to have been lower (N:P<15; Hessen and Lyche 1991). Therefore, a stoichiometric imbalance between *Daphnia* and seston was likely to be evident and positive, maybe even pronounced. Consequently, *Daphnia* growth caused P limitation in phytoplankton. In turn, in the Kiel Fjord experiment, N:P ratios of seston (N:P= 13 to 22) and of *A. clausi* (N:P~14 to 22; Gismervik 1997, Walve and Larsson 1999, Petrola et al. 2002) were probably within the same range. Hence, copepods resupplied phytoplankton with nutrients in the same ratio as required by phytoplankton, and thus did not negatively affect seston C:N or C:P ratios.

(2) Phytoplankton growth may only become limited by a specific nutrient, if its supply is directly dependent on zooplankton recycling. If phytoplankton can draw nutrients from other nutrient pools, nutrient retention in zooplankton will not affect phytoplankton cell quotas.

Such 'compensatory uptake' of nutrients probably occurred in the Kiel Fjord experiment, where the supply of both, P (from the DIP pool), and N (from N fixation by *N. spumigena*.) could easily 'compensate' for any elemental deficiency in the nutrient supply ratio of phytoplankton. Consequently, zooplankton had no negative impact on seston C:N or C:P ratios. In turn, inorganic P (SPR) in the Schöhsee experiment, was apparently bound to calcite ('whiting') and, therefore, not available to phytoplankton. Consequently, P retention in *Daphnia* growth (numerical increase) substantially depleted phytoplankton cell quotas for P, yet not for N, since *Daphnia* P demand was higher.

(3) Zooplankton need to be a quantitatively important nutrient compartment (Hessen et al. 1992).

As outlined previously, the peak density of juvenile *O. dioica* (35 ind L<sup>-1</sup>) in the Hopavågen experiment were estimated to represent ~13 to 20% and ~20 to 25% of total particulate N and P, respectively. Although a contribution of >20% P to total particle P is considered to have an impact on pelagic nutrient dynamics (Hessen et al. 1992), *O. dioica* evidently showed no effect on seston stoichiometry. A comparable copepod density in the Kiel Fjord experiment (38 ind L<sup>-1</sup>), represented ~30% and ~25% of total particulate N and P, respectively (assuming 1.5 µg N and 0.15 µg P ind<sup>-1</sup>, calculations for 13 September). One may speculate whether copepods, in the absence of *N. spumigena* or a depleted DIP pool, would have retained substantial amounts of N or P. In the Schöhsee experiment, where *Daphnia* proved to have a negative impact on seston C:P ratios, a similar density of *Daphnia* (35 ind L<sup>-1</sup>) constituted ~43 to 55% and ~57 to 68% of total particulate N and P, respectively (assuming 17 µg dry weight ind<sup>-1</sup>, 1.1 to 1.8% P content, body N:P = 14:1, calculations for 21 August). For *Daphnia*, the contribution to total particulate N and P was, hence, by a factor of 2 to 3 higher than for both, *O. dioica* and *A. clausi*. This shows that zooplankton must not only have a body stoichiometry that deviates from the stoichiometry of its food, but also be a quantitatively important sink for nutrients.

(4) Nutrients can only be retained in zooplankton (somatic and reproductive growth), if the C content of their food is above basic energy requirements (Urabe and Watanabe 1992).

Such basic C requirements were probably met for zooplankton over most of the density gradients in the Schöhsee and Kiel Fjord experiments. This was indicated by the fact that zooplankton increased in abundance over the experimental period, and by the relatively high seston C concentrations (~50 and ~80 µmol C L<sup>-1</sup>, respectively). The results for both experiments are therefore consistent with the predictions of ecological theory: P-limitation due to preferential P retention and N recycling (*Daphnia*), and the lack of nutrient limitation due to similar grazer-resource N:P ratios and/or a 'compensatory' supply of nutrients (*A. clausi*).

In the Hopavågen experiment, high mortality of copepods, even at low densities, indicated that copepod energy limitation was pronounced. Since zooplankton growth is a prerequisite for the concept of consumer-driven nutrient recycling, increases of the seston  $\Delta(C:N)_t$  ratios with copepod density cannot be explained by preferentially nutrient recycling. Instead, the responsible mechanism seemed to be the shift in the size structure of phytoplankton from diatoms to nanoflagellates mediated via a trophic cascade 'copepods-ciliates-nanoflagellates': increased nanoflagellate growth at the expense of the intracellular N pool resulted in increased seston  $\Delta(C:N)_t$  ratios. This enhanced phytoplankton N-limitation, irrespective of nutrient fertilisation from dead copepods.

Models of consumer-driven nutrient recycling are usually simplified to the nutrient dynamics of only three compartments (zooplankton, phytoplankton, dissolved inorganic nutrients). A three-box model may indeed be a valid approximation for the grazing of *Daphnia*. This is because *Daphnia* ingests particles over a wide particle size range, including bacteria and ciliates (Jürgens 1994). And, because *Daphnia* may spare only some large phytoplankton (e.g. dinoflagellates, Table 4) with a low compensatory growth capacity (Banse 1992) for the reduction of C concentrations in seston (Figure 16, Sterner 1989). Therefore, the reduction of *Daphnia* dietary particles (algae and microzooplankton) – measured here as seston – may approximate the reduction of phytoplankton biomass.

For marine oligotrophic systems, a realistic model would have to consist of at least four boxes, with intermediate consumers (e.g. ciliates) as an additional compartment. This is because copepods may prey heavily on ciliates (Kleppel 1993, Tables 5 and 7), and because nanoflagellates, as important primary producers, are beyond the edible size range of copepods. Consequently, the reduction of copepod dietary particles (mainly microzooplankton) may not result in the reduction, but - instead - in the increase of phytoplankton biomass (Figure 20). Although both, *Daphnia* and marine copepod grazing may similarly result in increased phytoplankton nutrient limitation, the responsible mechanism appears to be fundamentally different: *Daphnia* strongly controls both, C and P dynamics, whereas copepods in marine oligotrophic regions enhance C production by shifting phytoplankton size structure, thereby 'diluting' the intracellular N pools of phytoplankton. This same mechanism may, in fact, also apply for appendicularians. Recently, Fernández and Acuña (2003) showed that these zooplankton equally shift phytoplankton structure albeit to large diatoms, increasing CO<sub>2</sub> fixation.

It may be concluded that for both, nutrient-rich (Kiel Fjord) and nutrient-poor (Hopavågen) marine systems, the concept of consumer-driven nutrient recycling is not of great importance, as predicted from theoretical calculations (Walve and Larsson 1999, Petrola et al. 2002). Freshwater *Daphnia* in lakes, are simply different from copepods in the sea: They are effective grazers, stoichiometrically very different from their food, and big. And it is this difference that matters.

## VI. Appendix

### Do calanoid copepods suppress appendicularians in the coastal ocean?

Published in Journal of Plankton Research (2003) 25: 869-871

#### Abstract

In a mesocosm study, the appendicularian *Oikopleura dioica* bloomed after the decrease in numbers of copepods, and in a second treatment, showed a significantly negative correlation with copepod densities. Calculations, together with field data from the Baltic Sea suggest that common calanoid copepods may control appendicularian population dynamics.

#### Introduction

In marine ecology, appendicularians have received considerable attention for their ability to feed on small particles (Flood et al. 1992), and for processes associated with the sedimentation of their filtering devices, so-called 'filter houses' (Alldredge 1976). However, little is known about the factors that regulate the population dynamics of appendicularians. Generally, these have been associated with phytoplankton blooms (Valentin et al. 1987, Nakamura 1998) and water temperature (Acuña and Anadón 1992). Yet, high concentrations of appendicularians may occur with no evident correlation to the seasonality of phytoplankton (Landry et al. 1994). Also, predation by fish larvae has been speculated to control appendicularian population dynamics (Nakamura 1998), as appendicularians may constitute a high portion of their diets (Shelbourne 1962, Ryland 1964). Here, we discuss the possibility of grazing by common calanoid copepods to be an important factor in the control of coastal appendicularian population dynamics.

#### Methods

An enclosure experiment, originally designed to test the grazing impact of copepods on phytoplankton, was conducted in the sheltered semi-enclosed marine Hopavågen lagoon situated on the outlet of the Trondheim Fjord, Norway, in July 2001. Water temperatures were  $\sim 13^{\circ}\text{C}$ . The enclosure bags ( $\sim 1.5 \text{ m}^3$  volume) were filled by hauling the submerged bags to the surface from  $\sim 3 \text{ m}$  depth. Vertical net hauls with a  $250 \mu\text{m}$  plankton net were performed to decrease the numbers of mesozooplankton inside the bags. Thereafter, two sets of treatments were established: a copepod treatment (10 enclosure bags), and a copepod-reduction treatment (6 bags). For the copepod treatment, copepods were collected in the lagoon with a  $250 \mu\text{m}$  plankton net and added to 10 bags, at final concentrations of 5, 10, 20, 40 and  $80 \text{ copepods L}^{-1}$ . Each treatment density was duplicated. Copepods were primarily the common calanoid species *Temora longicornis* ( $\sim 45\%$ ), *Centropages hamatus* and *Centropages typicus* (together  $\sim 24\%$ ), *Pseudocalanus elongatus* ( $\sim 24\%$ ), and *Acartia longiremis* ( $\sim 5\%$ ). No copepods were added to the remaining 6 bags of the copepod-reduction treatment. Samples

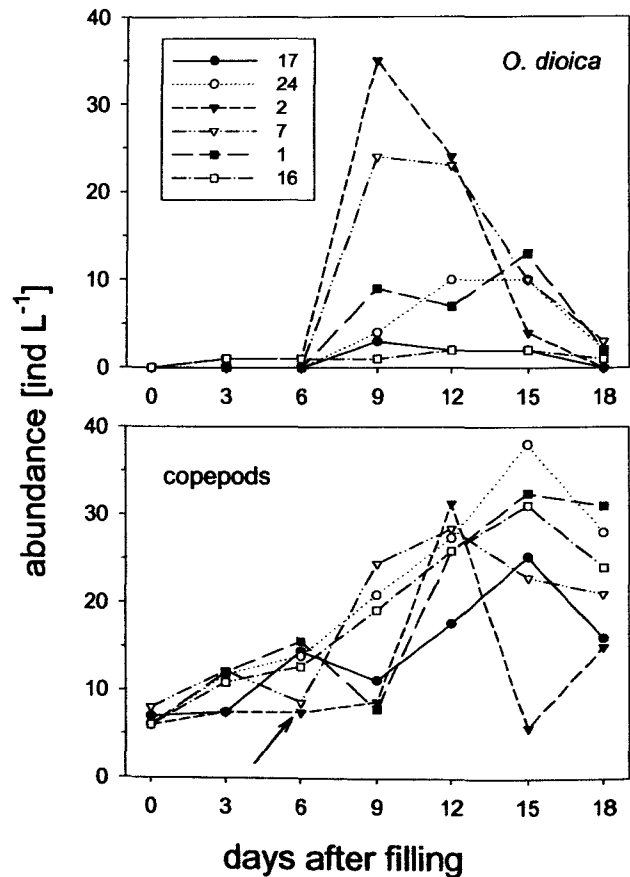


for zooplankton counts were taken every 3 days with a 50  $\mu\text{m}$  plankton net (contents of 12 L water volume) and preserved with formalin (4% final concentration). Subsamples of at least 200 total individuals were counted in order to determine zooplankton densities.

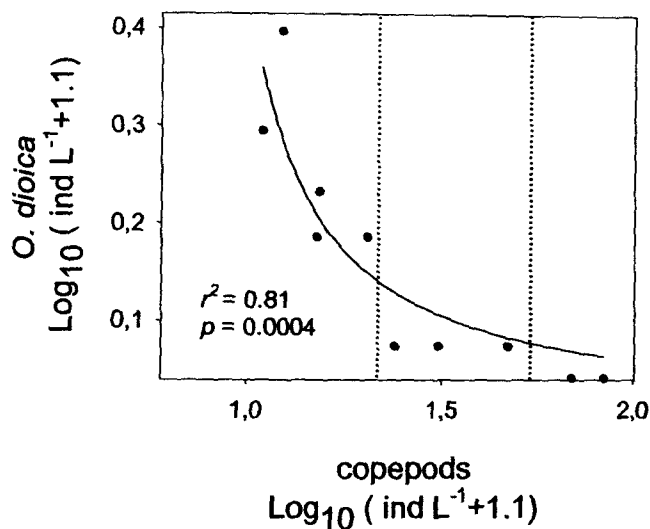
## Results and Discussion

In the copepod-reduction treatment, *O. dioica* developed 9 days after filling the bags, reaching peak densities of 24 and 35 ind  $\text{L}^{-1}$ , respectively (Figure 1). The appendicularian bloom lasted for roughly a week. Copepods were initially present at  $\sim 7$  ind  $\text{L}^{-1}$  and generally increased in abundance to  $>20$  to 30 ind  $\text{L}^{-1}$  along the course of the experiment. Notably, the highest densities of *O. dioica* on day 9 were observed in bags of lowest ( $<10$  ind  $\text{L}^{-1}$ ) preceding copepod abundance on day 6.

**Figure 1:** Copepod-reduction treatment: Abundance [ind  $\text{L}^{-1}$ ] of *O. dioica* and of copepods along the course of the experiment. Symbols represent the various enclosure bags. The arrow indicates bags of lowest copepod densities on day 6.



In the copepod treatment, *O. dioica* was present at very low densities ( $<2$  ind  $\text{L}^{-1}$ ) throughout the experimental period, but was generally larger in trunk length ( $>1$  to 2 mm) compared to individuals in the copepod-reduction bags ( $\leq 1$  mm) (data not shown). Plotted as a function of copepod abundance, appendicularians significantly decreased with increasing copepod densities, being practically absent at copepod densities  $>13$  ind  $\text{L}^{-1}$  (Figure 2). This absence of *O. dioica* at higher copepod densities was mirrored in the Hopavågen lagoon, where *O. dioica* was practically undetectable at the study site during July, when natural copepod abundance ranged from 23 to 55 ind  $\text{L}^{-1}$ .

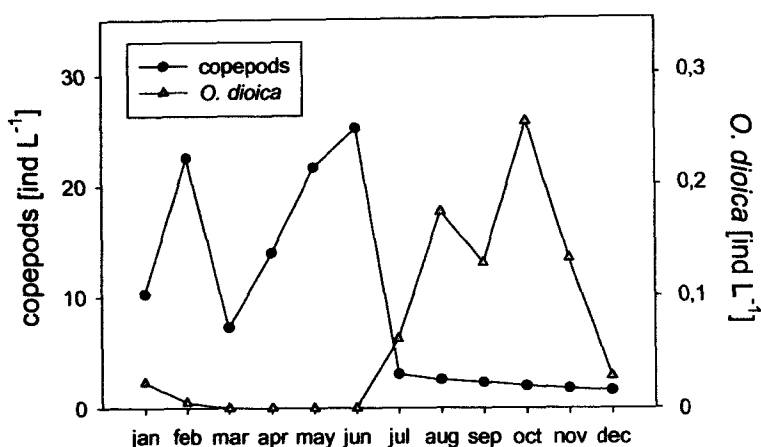


**Figure 2:** Copepod treatment: Abundance of *O. dioica* as a function of copepod abundance on day 6. A non-linear regression [ $y=1/(14.6x-12.4)$ ] was fitted to the  $\text{Log}_{10}$ -transformed data. The area between the dashed lines shows the range of natural copepod abundance (23 to 55 ind  $\text{L}^{-1}$ ) during the experimental period at the study site.

To our knowledge, predation of appendicularians by copepods has only been reported for the carnivorous *Candacia bipinnata* (Ohtsuka and Onbé 1989), but not for other common surface-dwelling copepods. Therefore, it is not surprising that copepods are not associated with appendicularian population dynamics, and their abundance even generally not determined in appendicularian-related studies (Uye and Ichino 1995, Nakamura et al. 1997, Tomita et al. 1999). However, given the wide variety of plankton taxa (diatoms, ciliates, even copepod nauplii and rotifers) that common calanoid copepods, such as *Temora*, *Centropages*, and *Acartia* species, have been shown to feed on (Paffenhöfer and Knowles 1980, Stoecker and Egloff 1987, Caparroy et al. 1998), appendicularians may well be assumed a potential prey for copepods.

This assumption seems especially reasonable for *O. dioica* eggs or recruiting young, as the size of *O. dioica* eggs ( $66 \pm 8 \mu\text{m}$ ,  $n = 8$ ; F. Sommer, unpublished data) and of newly hatched individuals ( $116 \pm 5 \mu\text{m}$ ; Paffenhöfer 1973) lies well within the size range of copepod prey. Assuming typical clearance rates of 50 to 100  $\text{ml ind}^{-1} \text{d}^{-1}$  (Stoecker and Egloff 1987), copepod densities of already 10 to 20  $\text{ind L}^{-1}$  in our mesocosm bags would be sufficient to clear the entire enclosed water body of appendicularian juveniles. This circumstance may explain the observed absence of *O. dioica* in the copepod treatment at copepod densities  $>13 \text{ ind L}^{-1}$ , and in the Hopavågen lagoon ( $>23 \text{ ind L}^{-1}$ ). The decrease in copepod grazing pressure, as at the onset of the copepod-reduction treatment ( $<7 \text{ ind L}^{-1}$ ), therefore probably allowed for the survival of some *O. dioica* juveniles, hatching from the large number of eggs typically produced (130 to 360 eggs  $\text{female}^{-1}$ ; Paffenhöfer 1973). Moreover, their small size ( $<1 \text{ mm}$ ) and the time lag of 9 days in mass appearance, corresponds well with the calculated time for spawning and recruitment of young *O. dioica* (8 to 12 days at  $13^\circ\text{C}$ ; Paffenhöfer 1973, 8 days at  $15^\circ\text{C}$ ; Uye and Ichino 1995) suggesting that *O. dioica* individuals were in fact newly hatched young.

Similarly, in the study of Koshikawa et al (1999) the appendicularian *Oikopleura* sp. developed 5 days after the onset of their mesocosm study. There, too, initial copepod abundance was low (11 ind L<sup>-1</sup>) and even declined to ~7 ind L<sup>-1</sup>. Higher temperatures (28°C) compared to our study (~13°C), probably resulted in the shorter development time of juvenile appendicularians (5 days), released from copepod grazing pressure. The initiation of a comparable bloom of *O. dioica* in a previous mesocosm study in the Hopavågen lagoon (Stibor et al. 2003) indicates that this phenomenon is indeed reproducible.



**Figure 3:** Seasonal variation of copepods and *O. dioica* in Kiel Bight, western Baltic Sea. Data points represent monthly mean densities [ind L<sup>-1</sup>] measured at 5 different stations from 1985 to 1992. Data are from Behrends (1997). Note different scaling for copepod and appendicularian abundances.

Field data at times also suggest that appendicularians bloom after the decline of, or at low copepod abundance. A field survey in Japanese Seto Inland Sea showed that appendicularians bloomed at densities of calanoid copepods (mainly *Paracalanus parvus*) <10 ind L<sup>-1</sup> (Nakamura 1998). More impressively, long-term data from Kiel Bight, western Baltic Sea (Behrends 1997), show that appendicularians appear regularly from July to November, when waters are stratified and water temperatures high, thereby "coinciding" with the general decline of copepod abundance to <5 to 10 ind L<sup>-1</sup> after spring and early summer conditions (Figure 3). Although these blooms may be equally explained by correlations with water temperature and/or chlorophyll a concentrations, it is proposed that grazing by common calanoid copepods, at least on appendicularian eggs and/or early juveniles, should not be discarded a possibly important (additional) factor in the control of appendicularian population dynamics, especially at times of stratification in temperate coastal seas.

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## List of abbreviations and symbols

Abbreviation	Long spelling	Remarks
‰	-	unit in mass spectrometry
$\delta$ ( $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ )	-	the ratio of two stable isotopes in relation to a standard
C	carbon	-
$^{12}\text{C}$ , $^{13}\text{C}$ , $^{14}\text{N}$ , $^{15}\text{N}$	-	stable isotopes of C and N, respectively
chl a	chlorophyll a	-
d	day	-
DIN	dissolved inorganic N	comprises $\text{NO}_3$ , $\text{NO}_2$ and $\text{NH}_4$
DIP	dissolved inorganic P	in limnology: SRP
h	hour	-
ind	individual(s)	used in: $\text{ind L}^{-1}$
$n$	-	statistics: sample size
no.	number	-
N	nitrogen	-
$\text{N}_2$	binitrogen	-
$\text{NH}_4$	ammonia	correctly: $\text{NH}_4^+$
$\text{NO}_2$	nitrite	correctly: $\text{NO}_2^{2-}$
$\text{NO}_3$	nitrate	correctly: $\text{NO}_3^{2-}$
$p$	-	statistics: probability of an observation
P	phosphorous	-
$\text{PO}_4$	phosphate	correctly: $\text{PO}_4^{3-}$
POM	particulate organic matter	here: equivalent to the term 'seston'
PON, POP	particulate organic N, P	here: equivalent to seston N, seston P
$r^2$	-	statistics: strength of a fitted regression
SD	standard deviation	statistics: measure of variability
SRP	soluble reactive P	in marine sciences: DIP
TN	total N	here: DIN, DON and PON excluding zooplankton
TP	total P	here: DIP and POP excluding zooplankton

## Acknowledgements

I would like to greatly thank Prof. Dr. Ulrich Sommer, who supervised this thesis and gave me the opportunity of learning about zooplankton and pelagic food webs. I am grateful to the personnel of the IfM, especially Harald Vogt, Ursula Frank-Scholtz, Volker Martens and Ulrich Stephan, who helped so much with the logistics and the construction of the floats used in the Baltic mesocosms. Many thanks to Jens Feiertag, head of the 'Wasser- und Schifffahrtsamt Lübeck' in Kiel-Holtenau, for his kind support during the experiment at the facilities. I am grateful to Alexandra Neyts (University of Trondheim) for help with the Norwegian toll, and to Jette Dröse ('100 ml gehen noch'), Kerstin Maczassek, Jennifer Netzer, Eckart Zöllner and Julia Wohlers for friendly support during field work. Many thanks to my friends and colleagues Thomas Hansen, Sybille Jaschinski, Daniela Brepohl, Steffi Moorthi and Marcel Sandow, who helped with many things: analytics, species determination and comments on an earlier version. I would also like to thank the Deutsche Forschungsgemeinschaft for employment and financial support (projects SO 145/19-1 and SO 145/19-2). Finally, I wish to thank my mother, who supported me during the years of my studies, and Dorothea, who endured my moods and gave me strength during this period.